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CARBON DIOXIDE EXCHANGE RHYTHM AND FRUITFULNESS IN PLANTS OF DIFFERENT REPRODUCTIVE HABITS [1]

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INTRODUCTION

The proposition that sexual reproduction in plants results from like physiological conditions in different species is not new. Such chemical work as that of Kraus and Kraybill (*3*) [3] on the relation of the nitrogen and carbohydrate compounds to reproduction is based upon this concept. They called attention to the definite relation between the vegetative condition of plants and blossoming and fruiting. This relation has been repeatedly demonstrated in recent years,[4] but at the same time the possibility of chemical content being correlated with reproduction has been questioned almost or quite as often as it has been asserted. Conflicting data have left the issue unsettled. The belief that the fruiting condition of the plant can be measured by present chemical methods was questioned several years ago (*4*). Until it is possible to extract and measure either morphological or physiological fractions of the plant, chemical analyses as commonly applied would appear to have a relatively limited value in any attempt to determine the physiological basis for reproduction phenomena.

If present chemical methods are unsatisfactory, what methods can be applied to measure and record the very apparent relation of the growth character of the plant to reproduction? One method has proved satisfactory; that is, to determine the relation of anatomical characters and cambial condition to the reproductive stage. Examination of a number of species has disclosed that what may be said to be comparable anatomical conditions precede or accompany flowering in these plants, although widely different environmental conditions are required to induce blossoming (*7*). The present paper is a report of the records and observations obtained in studying the carbon-dioxide exchange of 15 species of plants having widely different reproductive habits.

Some years ago it was thought that studies of CO_2 exchange might throw light upon the obvious discrepancy between the cultural treatment of some plants and their carbohydrate accumulation. For example, plants of some species show relatively slight accumulation when grown under good light conditions and supplied with abundant water and nutrient high in nitrogen but may show relatively large

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[3] Reference is made by number (italic) to Literature Cited, p. 343.
[4] ROBERTS, R. H. THE CARBOHYDRATE-NITROGEN QUESTION. Paper presented at meeting of Amer. Soc. of Plant Physiol., St. Louis, December 1935.

accumulation when grown in shade and given little or no nitrogen. The accumulation of carbohydrates is conditioned, of course, by utilization as well as by synthesis. From the standpoint of carbohydrate source it was desired to measure the CO_2 intake of plants receiving different cultural treatments. Such measurements are given in tables 1 to 6. These show that the apparent CO_2 intake is conditioned by the "internal environment" of the plant, as a result of its previous history, as well as by the immediate cultural conditions. The so-called balance within the plant affected the CO_2 exchange as much as it had previously been found to modify the effects of other factors upon

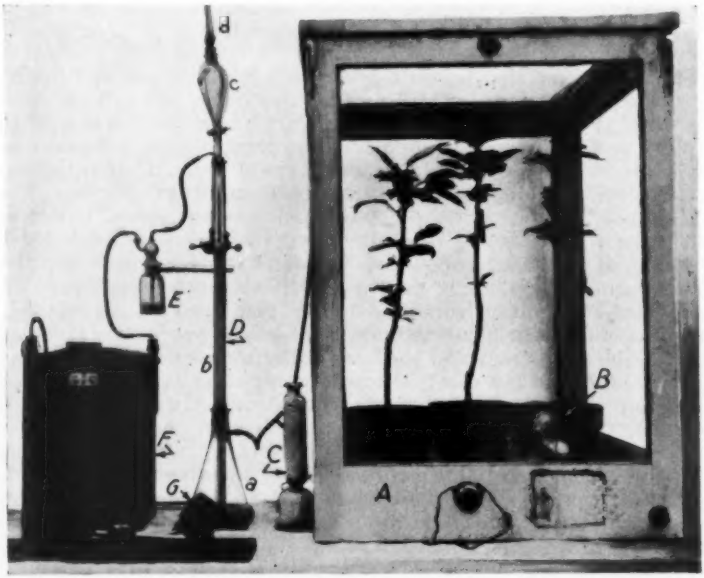

FIGURE 1.—Arrangement of plant chamber and absorption system. Manometer is not shown. A duplicate chamber stands at left of air meter. *A*, Chamber (containing Tetofsky apple trees); *B*, fan to circulate air; *C*, soda-lime tower to supply CO_2-free air to absorption tower; *D*, absorption system; *a*, suction flask; *b*, glass tube containing glass beads; *c*, separatory funnel for alkali; *d*, chloride tube with soda lime; *E*, wash-bottle trap; *F*, air (gas) meter; and *G*, suction pump driven by electric motor.

such a character as blossoming (*5*). Thus the amount of CO_2 intake during the day seemed no more the direct cause of a particular plant performance than other factors such as nitrogen nutrient, shade, etc. It was noted, however, that flowering and nonflowering plants of some species had different CO_2 exchange curves or rhythms. This raised the question as to whether reproductive and nonreproductive plants are characteristically different physiologically as indicated by CO_2 exchange, and led to the collection of the data (figs. 9–28) with which the present paper is principally concerned.

METHODS OF EXPERIMENTATION

Large plant chambers of approximately 30 cubic feet capacity (fig. 1) were used in preference to single leaf chambers (*2*). This method permitted the use of several whole plants in a sample, thus reducing

any error due to variations in plants or plant parts. Sufficient numbers of plants were included in a sample to give a total of more than 200 square inches of leaf surface. As a rule not more than 600 square inches were used. The data are presented as milligrams of CO_2 exchanged per hour per 100 square inches of leaf area. The recorded values represent aliquots of about one-tenth to one-eleventh the total amount of CO_2 used or given off, since the 3 feet passed through the absorption towers was approximately this part of the total capacity of the chambers plus 3 feet of added air to replace that drawn from the chambers. This gives the relative amounts of CO_2 exchange of two sets of plants at a given time and does not, of course, measure the exact quantity of gas involved.

The usual procedure when taking a sample is to place the plants in the chambers, close the chambers, fill the absorption towers with CO_2 free air, add 50 cc of N/10 $Ba(OH)_2$ from the separatory funnels and then sufficient water to cover the glass beads during suction. The pumps are then started and the manometers adjusted to pass 3 cubic feet of air as measured by the gas meters in approximately 2 hours. When 3 feet of air has passed the towers, the absorption system is closed, suction stopped, and the beads rinsed into the Erlenmeyer flasks with CO_2—free water. The plant chambers are opened and aerated until the next sample is to be collected, a period of about an hour.

The amount of CO_2 absorbed was determined by titration, phenolphthalein being used as an indicator. This method was employed in the early trials and was continued even after recent methods were announced, as it seemed best suited to the conditions of the experiments and the measurement of the low dilutions of CO_2 with which the writers worked.

The air in the chambers was circulated continuously during the tests by small electric fans.

Especial care was taken to secure an accurate measurement of the volume of air passing the absorption towers. The meters were frequently recalibrated and were found to be dependable for rates of flow as small as one-quarter of that used. To further insure against variable data due to possible development of meter trouble, the meters serving the two plant chambers were alternated on successive days.

The use of whole plants in pots introduced the apparent error of having soil in the chambers. Measurements showed that the CO_2 change due to the soil was very small as compared to that due to the plant foliage. Even if the soil had caused more than a negligible error it would be compensated for, as equal numbers of pots and so like amounts of soil were present in the two plant chambers at the same time.

It is particularly necessary to avoid contaminating the air of the plant chambers by the operator's breath.

Equal and uniform humidity was secured by keeping the potted plants in shallow saucers of water.

As plants were being grown in the greenhouse where the chambers were located, a constant change in the CO_2 content of the air was taking place, especially in cold weather when there was little ventilation. This necessitated the determination of the amount of CO_2 in the air enclosed in the chambers at the beginning of each 3-hour period.

Temperature corrections were not made since the temperatures within the chambers were comparable. More important, however, is the very apparent fact that temperatures between 15° and 35° C. had relatively little effect upon the CO_2 exchange curves (figs. 9–28) under

the conditions of the experiment. It would appear that respiration changes due to temperature largely compensated for temperature effects upon CO_2 movement. CO_2 utilization appeared to be reduced on the few occasions when extreme temperatures of near 40° were encountered. Temperatures below 15° were avoided as being unsatisfactory for growth.

It became evident in the early work that the respiration rate affected the CO_2 uptake during the daytime, and it was therefore necessary to make determinations during or throughout the night if the daytime data were to be properly interpreted. Consequently, readings were made on many sets of plants at 3-hour intervals during periods of from 1 to 6 days.

The daytime determinations were made under natural light conditions. The short-day plants were exposed from 8 a. m. until 4 p. m., and the long-day plants had added light from about an hour before sunset until midnight. The additional light was supplied by one 250-watt lamp suspended above the plant chamber. This amount of light was sufficient to maintain long-day responses of the plants and did not give off enough heat markedly to change the temperature within the large chamber. While the requirements vary with the variety of the plant, minimum light conditions for optimum CO_2 uptake during the day are approximately those of a hazy or light cloudy period. Dark clouds usually reduce the CO_2 intake of plants, some species such as celery being an exception. The amount of sky and sun radiation as recorded at a nearby station of the United States Weather Bureau is shown in figures 9 to 28. The values indicated are the 2-hour totals during the time of sampling as measured by a calendar pyrheliometer and recorded as gram-calories.

Under the atmospheric conditions at Madison the Bureau measurements are applicable to the greenhouse location.

When two branches of a single plant were to be used, cellophane chambers having a cubic content of about 7 feet were substituted for the glass chambers shown in figure 1. Smaller leaf areas were used so that the CO_2 concentration of the air per leaf area was comparable to that in the tests made with the larger chambers. The recorded values, however, are not comparable, as the 3-foot sample used is a larger aliquot of the air from the smaller chambers.

Probably the most significant remark to be made regarding the technique employed is that it produced CO_2 exchange curves of striking smoothness and symmetry, as illustrated by the performance of the nonflowering poinsettia plants shown in figures 9–14.

EXPERIMENTAL MATERIAL

The following plants were used in the CO_2 utilization determinations: Apple (*Malus sylvestris* Mill.), buckwheat (*Fagopyrum esculentum* Moench), hybrid calceolaria (*Calceolaria fruticohybrida* Voss), celery (*Apium graveolens* L. var. *dulce* DC.), Klondike cosmos (*Cosmos sulphureus* Cav.), jimsonweed (*Datura stramonium* L.), geranium (*Pelargonium domesticum* Bailey), goldenglow (*Rudbeckia laciniata* var. *hortensia* Bailey), pigweed (*Amaranthus retroflexus* L.), poinsettia (*Euphorbia pulcherrima* Willd.), potato (*Solanum tuberosum* L.), soybean (*Soja max* (L.) Piper), spinach (*Spinacia oleracea* L.), ornamental sunflower (*Helianthus multiflorus* Hort.), and Maryland Mammoth tobacco (*Nicotiana tabacum* L.)

The early studies of the influence of environment upon CO_2 utilization were made with apple trees. The effect of varying the nitogen nutrient, photoperiod, stock variety (fig. 2), and shade were studied. Tests were also made to determine the effect of girdling young trees early in the growing season upon their CO_2 utilization later in the season.

Most of the fruiting and nonfruiting plants of the later series were secured by modifying the photoperiod.

The fact that a long or short daily light period may hasten flowering does not mark a plant as a "long-day" or "short-day" kind. Unless it will blossom only under long- or short-day conditions it should be called indeterminate (*1*). In a search for the physiological basis for photoperiodicity the tendency to speak of the environment and the responses of the plant as interchangeable should be avoided. The

FIGURE 2.—Winesap apple trees in bank sand (partial defoliation due to seasonal maturity): *A*, *B*, With added nitrogen and grafted on Wealthy and York Imperial; *C*, *D*, without added nitrogen and grafted on Wealthy and York Imperial.

question is not, do certain plants which are grown under long- and short-day conditions have a different or similar chemical composition, anatomical situation, or other character of plant at a given time, but rather, do plants in a truly different reproductive condition have a different or similar composition.

Poinsettia appears to be a specifically short-day plant (fig. 3); so much so, that separate branches of a single plant gave characteristic fruiting responses when grown under different photoperiod conditions (fig. 4).

Indeterminate types are often erroneously classified as long- or short-day plants since they may blossom earlier in one environment

than another, although they will fruit in both after different lengths of time, datura (fig. 5, *E* and *F*) for example, fruits earlier when grown in a short day, but will eventually fruit under long-day conditions.

The difficulties involved in making photoperiod classifications are well illustrated by the reaction of *Helianthus multiflorus* Hort. This plant did not flower when grown under short-day conditions, but when given an initial period of long-day culture and then transferred to short-day conditions it soon blossomed. (Fig. 6).

Potato plants make a marked response to photoperiod conditions (fig. 7). In the case of Triumph, the plant is dwarfed and tubering is hastened by short days. Neither lot shown in figure 7 produced blossoms.

Maryland Mammoth tobacco required a short photoperiod to initiate blossoming but also needed a low nitrogen nutrient in order to set its blossoms under the short-day conditions provided (fig. 8).

FIGURE 3.—Poinsettia: *A*, Long-day, nonfruiting; *B*, short-day, fruiting; *C*, fruiting as in *B* but rendered vegetative by change to a long-day environment; *D*, older vegetative plant rendered reproductive by short-day environment.

EXPERIMENTAL RESULTS

The data from the first lot of apple trees are given in table 1. These were principally trees that had had different quantities of nitrogen. Sand culture plus mineral nutrient was used. Yearling trees were grown 2 years in pots out of doors and the third season started in the greenhouse in January for the CO_2 exchange measurements.

Tables 2 to 4 give the data secured on apple trees during their first season in pots. The culture conditions are indicated in the tables. The original trees were 2 years old and were grown out of doors. The carbon dioxide measurements given in series 2, table 2, were made under natural light conditions; that is, trees grown under short-day conditions were sampled under prevailing daylight conditions.

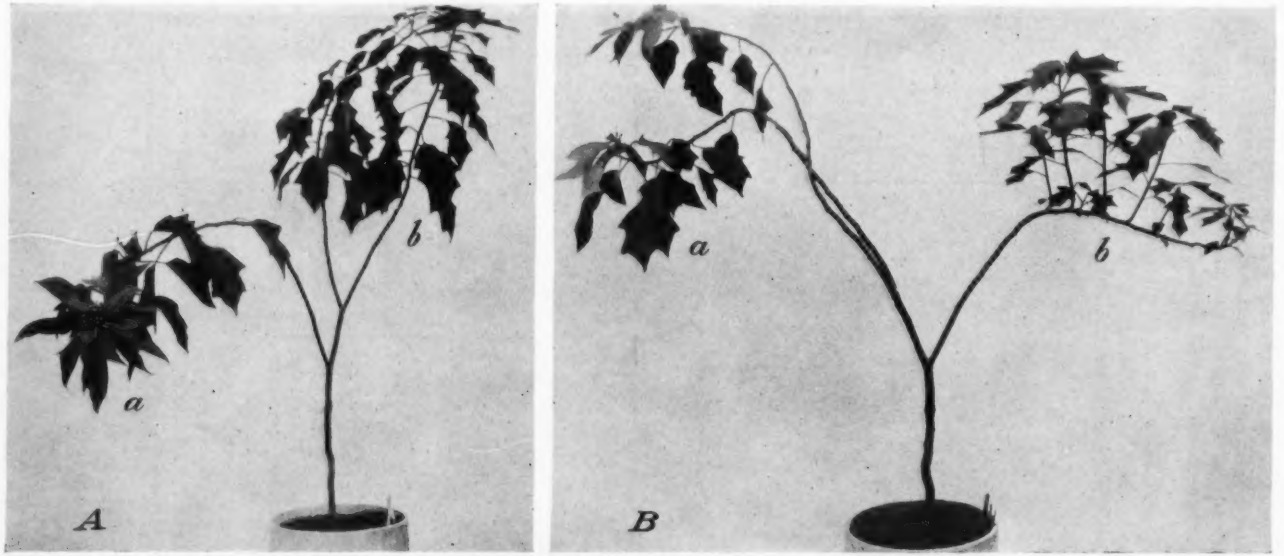

FIGURE 4.—Poinsettia: *A*, Fruiting, short-day (*a*) and nonfruiting, long-day (*b*) branches of same plant; *B*, plant similar to *A* after reversed treatments, showing flowering branches at *a* after change to short-day, and vegetative growth at *b* under long-day conditions on branch formerly reproductive.

Table 2, series 3, shows the carbon dioxide utilization of apple trees in reduced light and also under short-day conditions (light from 8 a. m. until 2 p. m.). Lot 27 was shaded with 8-ounce burlap and lot 28 with cheesecloth. Lots 29–31 were short-day trials without shading.

Table 3 records the effect of girdling apple trees in the early stages of growth upon the CO_2 exchange in the later growth season.

The CO_2 exchange of trees grafted on different stocks is shown in table 4.

FIGURE 5.—*A*, Long-day, fruiting rudbeckia; *B*, short-day, nonfruiting rudbeckia obtained by transferring long-day plants to short-day environment; *C*, long-day, fruiting spinach; *D*, short-day, nonfruiting spinach; *E*, long-day, nonfruiting datura; *F*, short-day, fruiting datura.

The Wealthy trees of table 5 were started in January 1934 in the greenhouse under normal winter light, and long- and short-day treatments were begun March 5. At the time of sampling the long-day trees were making much faster growth than the short-day trees. Soil culture was used.

The potted apples of 1933 were replaced in pots with bank sand in February 1934 and grown in natural light. Nitrogen nutrient was added. The effect of the nutrient on the utilization of carbon dioxide is shown in table 6.

FIGURE 6.—*Helianthus multiflorus* grown from cuttings: *A*, Longday, beginning to flower; *B*, 30 days in short-day environment after being in long-day environment fruiting; *C*, short-day, nonfruiting.

FIGURE 7.—Triumph potato plants grow less and form tubers earlier in a short-day environment (*A*) than in a long-day environment (*B*).

FIGURE 8.—Maryland Mammoth tobacco: *A*, Long-day, nonfruiting; *B*, short-day, reproductive, blossoms abscissing; *C*, short-day plant as in *B* grown in soil and becoming vegetative; *D*, short-day plant as in *B*, grown in bank sand and setting fruit.

TABLE 1.—*Carbon dioxide utilization by apple trees in series 1*[1]

Variety	Culture			Date sampled 1933	Weather		Time of maximum temperature	CO_2 at hour indicated								
	1931; nitrogen	1932; light	1933; nitrogen		Light	Temperature range		6 p. m.	9 p. m.	12 p. m.	3 a. m.	6 a. m.	9 a. m.	12 m.	3 p. m.	6 p. m.	9 p. m
						°C.		*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
Wealthy	Plus	Shade	Plus	May 15–16	Clear	17–36	Noon	+0.21	+0.26	+0.21	+0.52	−0.30	−2.37	−0.70	−1.25	+0.37	
	Minus	Sun	do					−.60	+1.01	+1.26	+1.92	+.89	−2.30	−2.42	−.40	+1.20	
Wealthy	Plus	do	Minus	May 17–18	Cloudy	19–31	do	+.01	+1.53		+.68	−1.28	−3.29	−2.36	−1.92		
	Minus	do	do					+.94	+3.14	+4.51	+4.39	+2.41	−1.33	−1.62	−.55		
Wealthy	Plus	do	do	May 22–23	Clear	18–37	do	−.50	+1.95	+1.51	+1.31	−1.32	−3.64	−2.31	−1.72		
York Imperial	do	do	do					+.09	+2.33	+2.23	+2.14	−.47	−2.78	−1.78	−1.25		
Wealthy	Minus	do	Plus	May 24–25	do	17–42	do	+.39	+1.86	+2.45	+1.17	+.53	−3.48	−3.52		+.84	+1.77
York Imperial	do	do	do					+.71	+2.57	+1.74	+1.68	+.38	−2.13	+.12	+.25	+1.52	+2.49
Wealthy	Plus	do	do	May 26–27	Cloudy	17–33	9 a. m.	+.38	+.50	+.48	+.57	−.62	−3.43	−1.41	−.55		
York Imperial	do	do	do					+.01	+.91	+.72	+.84	−.44	−2.29	−1.53	−.43		
Wealthy	Minus	do	Minus	June 1–2	Clear	19–41	Noon	+.63	+1.41	+2.87	+2.73	−.05	−2.43	−3.00	−1.56		
York Imperial	do	do	do					−.31	+2.45	+2.79	+2.50	+.36	−.85	−.82	+.01		
Tetofsky	Plus	do	Plus	June 16	do	24–31	do					−.51	−3.57	−1.96	−.85	+.66	
York Imperial	do	do	do									−.02	−1.91	−.94	−.57	+.12	
Northern Spy	Minus	do	Minus	July 5	do	23–41	do					−.90	−1.56	−1.15	−.82	−.47	+.31
York Imperial	do	do	do									−.09	−.67	−.39	−.06	+.24	+.98
Northern Spy	Plus	do	do	July 7	Cloudy	26–34	do					−1.35	−3.11	−2.07	−1.82	−1.49	−.20
Tetofsky	do	do	do									+.10	−1.09	−.23	+.21	+.64	+.65
Wealthy	do	do	do	July 11	Clear, cloudy	23–36	do					−.04	−2.74	−1.47	−1.02	+.41	
Tetofsky	do	do	do									+.04	−1.30	−.38	+.04	+1.08	

[1] Results given as milligrams of CO_2 per hour per 100 Square inches of leaf area. Minus values show CO_2 extracted from the air, and plus values, CO_2 added to the air. Values are aliquots and are approximately one-tenth the actual utilization.

TABLE 2.—*Carbon dioxide utilization by apple trees of series 2 and 3, 1933*[1]

SERIES 2

Experiment or lot no.	Variety	Culture		Date	Weather		Approximate time of maximum temperature	CO_2 at hour indicated				
		Nitrogen	Light		Light	Temperature range		3 a. m.	9 a. m.	12 m.	3 p. m.	6 p. m.
						°C.		*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
2	Wealthy	Minus	Sun	July 28	Clear	23–38	Noon	0.00	−1.28	−0.78	+0.24	+0.77
			Shade					+.65	−.69	−.46	−.33	+.24

	do	do	Sun	Aug. 8	do	22-36	do	+.32	−1.97	−1.43	−.32	+.04
			Short					+.95	−2.66	−1.77	−.78	+.11
									−1.33	−2.75	−.92	−.73
7	do	Plus	do	Sept. 14		17-24	do	−1.19	−.88	−2.25	−.52	
		Minus	do					−.65	−1.62	−1.73	−1.52	+.13
6	do	Plus	Sun	Aug. 10	Hazy, clear	21-37	do	−.94	−1.91	−2.28	−2.07	+.26
	York Imperial	do	do					+.83	−1.33	−2.02	−1.38	+.58
	Wealthy	do	do	Oct. 19	Cloudy	15-26	do	+.18	−1.08	−1.77	−1.20	−.14
26	York Imperial	do	do					+1.46	−2.69	−2.86	−1.20	+.69
		do	do	Oct. 4	Clear	15-28	do	+1.47	−1.59	−2.18	−1.44	+.08
15	do	Minus	do						−1.82	−1.92	+1.08	+1.77
		Plus	do	Oct. 11	do	15-29	do		−1.99	−2.36	−.87	−.05
20	Tetofsky	Minus	do					+.84	−2.32	−2.58	−2.04	+.87
		Plus	Short	Oct. 5	do	15-30	do	+.61	−1.48	−2.13	−1.19	+.11
16	do	Minus	do					+1.42	−.50	−.12	−1.21	+.39
		Plus	Shade	Sept. 26	Cloudy, clear	15-24	3 p. m.	+.08	−.62	−.47	−.97	+.34
9	do	Minus	do					.00	−.78	−2.14	−1.57	+1.22
	do	Plus	do	Oct. 13	Clear	14-28	Noon	+1.09	−1.71	−2.51	−1.91	+1.93
22	Northern Spy	do	do					+1.81	−1.54	−2.41	−1.24	.00
	Tetofsky	Minus	do	Oct. 18	Clear, cloudy	14-28	do	+.74	−1.42	−1.62	−1.40	+.34
25	Northern Spy	do	do					+.31	−1.77	−2.58	−1.03	+1.17
		Plus	Short	Oct. 7	Cloudy, clear	13-29	9 a. m.	−.28	−1.48	−2.61	−1.48	−.27
18	do	Minus	do						−1.24	−1.04	−.61	+.39
		Plus	Sun	Oct. 2	Clear, cloudy	16-32	Noon	+.24	−1.28	−1.23	−.84	+1.72
13	do	Minus	do					+1.41	−2.22	−1.56	−1.49	+1.03
		Plus	Shade	Sept. 27	Clear	15-30	do	−1.30	−1.38	−.72	−.45	+.50
10	do	Minus	do					−.14	−1.06	−1.04		+.47
	do	Plus	Sun	Oct. 14	do	15-30	do	+2.72	−1.64	−2.03		+1.34
23	Whitney	do	do					+1.27	−1.10	−2.55	−1.75	+.37
		do	Short	Oct. 6	Cloudy	14-26	do	+.86	−2.15	−2.15	−1.68	−.35
17	do	Minus	do					−.18	−2.17	−2.11	−.63	+1.01
		Plus	Sun	Sept. 29	Clear	12-32	do	+.13	−1.91	−1.32	−.47	+1.26
12	do	Minus	do					+.20				

SERIES 3

27	Wealthy	Plus	Sun	Oct. 20	Cloudy, clear	17-25	Noon	+1.05	+0.35	−0.04	+.54	+1.18
			Shade					+.20	−.09	−.51	−.08	+.24
			Sun	Oct. 23	Clear, cloudy	15-20	do	+.19	−1.50	−1.93	.00	+1.05
28	do	do	Shade					−.83	−1.27	−.87	−.46	−.06
			Sun	Oct. 24		16-19	do	+.29	−2.14	−1.34	+.98	+.60
29	do	do	Short					−1.93		−2.15	−.03	+.19
			Sun	Oct. 25	Clear	16-27	do	+.96	−1.51	−1.46	+1.51	+1.28
30	do	Minus	Short					+.88	−2.57	−1.33	−.06	+.41
	do	Plus	Sun	Oct. 26	Very cloudy	15-17	3 p. m.	.00	−.96	−1.22	+.71	+.46
31	York Imperial	do						−.02	−.63	−.95	+2.57	+1.12
	Wealthy	Minus	do	Oct. 27	Cloudy, clear	15-23	Noon	−.07	−.82	−.63	+.85	+.42
32	Northern Spy	do						+.91	−1.08	−1.45	+2.59	+1.46

[1] See note 1, table 1.

TABLE 3.—*Carbon dioxide utilization by Wealthy apple trees of series 4, nitrogen nutrient, 1933, trees girdled in June* [1]

Lot no.	Light	Treatment	Date	Weather		CO_2 at hour indicated				
				Light	Temperature range [2]	6 a. m.	9 a. m.	12 m.	3 p. m.	6 p. m.
					°C.	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
1	Sun	Check	July 25	Clear	21–35	−0.45	−1.36	−1.45	−0.66	+0.47
		Girdled				+.87	+.20	−.54	+.17	+.97
21	do	Check	Oct. 12	do	15–28	+1.24	−2.19	−1.51	−1.55	+.16
		Girdled				+1.35	−.85	−.39	+.02	+1.00
3	Shade	Check	Aug. 1	do	24–38	+.63	−.70	−.48	−.81	+.72
		Girdled				−.11	−1.91	−1.27	−.45	+.16
8	do	Check	Sept. 25	Light cloudy	20–32	.....	+.02	−.23	−.65	+.54
		Girdled				+1.51	−1.83	−1.04	−.45	+.65
24	do	Check	Oct. 17	do	15–24	+2.69	−2.00	−2.17	−.40	+1.19
		Girdled				+.29	−.73	−.92	−.05	+.39

[1] See note 1, table 1. [2] Maximum always at noon period.

TABLE 4.—*Carbon dioxide utilization of long-day trees that had been grafted on stock of different varieties and given nitrogen nutrient, 1933* [1]

Lot no.	Variety	Stock variety	Date	Weather		Time of maximum temperature	CO_2 at hour indicated				
				Light	Temperature range		6 a. m.	9 a. m.	12 m.	3 p. m.	6 p. m.
					°C.		*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
4	Winesap	Wealthy, York Imperial.	Aug. 4	Clear	18–34	Noon	+0.12	−1.36	−0.94	−0.39	+0.1
							+.57	−1.80	−1.43	−.93	+.63
19	do	Wealthy, York Imperial.	Oct. 10	Clear, cloudy.	14–29	9 a. m.	+.84	−1.23	−1.19	−.09	+.36
							+1.24	−2.69	−3.04	−.06	+2.09
14	Yellow Transparent.	Fameuse (Snow), Whitney.	Oct. 3	Clear	13–32	Noon	+2.72	−1.06	−1.04	−.35	+.47
							+1.27	−1.64	−2.03	+.03	+1.34

[1] See note 1, table 1; also fig. 2.

The Wealthy trees of table 5 had been started in January 1934 in the greenhouse under normal winter light. The long- and short-day treatments were begun March 5. At the time of sampling, the long-day trees were making a much faster growth than the short-day trees. Soil culture was used.

TABLE 5.—*Effect of length of day upon carbon dioxide exchange of Wealthy short-day apple trees in daylight 8 a. m. until 4 p. m. and long-day trees exposed to added light until midnight* [1]

Light day	Date	Weather		Time of maximum temperature	CO_2 at hour indicated							
		Light	Temperature range		9 a. m.	12 m.	3 p. m.	6 p. m.	9 p. m.	12 p. m.	3 a. m.	6 a. m.
			°C.		*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
Long	Mar. 14	Clear, hazy	16–36	Noon	−0.83	−0.46	.....	.....	.....	.....	.....	.....
Short					+.38	−.07	−0.18	+2.39	+3.45	+1.99	+2.45	+1.35
Long	Mar. 15	Clear	16–32	do	−.90	−2.19	−1.33	−.39	+1.04	+.81	+1.14	+.51
Short					.....	−1.85	−.98	+1.15	+2.14	+1.82	+.89	+1.41
Long	Mar. 17	Dark, hazy	16–17	3 p. m.	+3.05	+.58	−1.21	+.07	.....	.....	.....	.....
Short					+3.94	+.34	−1.28	+1.30	.....	.....	.....	.....
Long	Mar. 19	Clear	17–35	Noon	−1.57	−1.55	−1.28	−.73	+.35	+.32	+1.66	+1.52
Short					−1.66	−2.47	−1.61	+2.00	+3.65	+1.85	+1.81	+.09
Long	Mar. 20	.....	.....	.....	−1.29	.....	.....	.....	.....	.....	.....	.....
Short					−.70	.....	.....	.....	.....	.....	.....	.....

[1] See note 1, table 1.

TABLE 6.—*Effect of variety and nitrogen nutrient upon carbon dioxide utilization by potted apples, 1934*[1]

Variety	Nitrogen	Date	Weather		CO_2 at hour indicated							
			Light	Temperature range[2]	9 a. m.	12 m.	3 p. m.	6 p. m.	9 p. m.	12 p. m.	3 a. m.	6 a. m.
				° C.	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
Wealthy	Plus	Apr. 11	Cloudy, hazy.	14-20	−0.94	−1.73	−0.20	+1.55	+3.43	.....	+1.70	+2.50
Do	Minus				−1.97	−3.20	−1.05	+.69	+1.69	.....	+1.42	+.98
Do	Plus	May 10	Cloudy	16-30	−.27	−.46	+.55	+.39	+.43	.....	.....	.....
Do	Minus				−.28	−.68	+.36	+.55	+.48	.....	.....	.....
Tetofsky	Plus	May 17	Clear	20-39	−.26	−.21	+.52	+.75	+1.00	.....	.....	.....
Do	Minus				+.54	+.62	+.97	+.53	+.50	.....	.....	.....
Do	Plus	Apr. 16	...do...	14-34	−1.39	−.53	−.04	+.59	+1.46	+2.02	+2.20	+1.44
York Imperial	...do...				−2.04	−.69	−1.78	+.21	+.98	+1.76	+1.23	+.91
Tetofsky	Minus	Apr. 26	...do...	15-31	−1.42	−1.33	−1.27	−.78	+.72	+.85	.....	.....
Whitney	...do...				−1.15	−1.40	−1.23	−.41	+.42	+.35	.....	.....
Do	Plus	Apr. 17	...do...	18-36	−1.83	−1.25	−.58	+1.13	+1.31	+1.10	.....	.....
Northern Spy	...do...				−.90	+1.00	+1.65	+.62	+3.89	+3.98	.....	.....
Do	Minus	May 3	Clear, hazy.	20-40	−1.00	−.44	−.47	+.37	+.91	+.77	.....	.....
Wealthy	...do...				−.63	+.07	−.21	+.31	+.58	+.76	.....	.....

[1] See note 1, table 1.

[2] Maximum always at noon period.

The remaining data, which are concerned with the utilization of CO_2 by flowering and nonflowering plants, were collected for the purpose of testing the theory that blossoming plants have a more irregular CO_2 exchange curve than nonblossoming plants. It is clear from the previous data that CO_2 exchange is not correlated with a particular external environmental factor but, rather, is associated with the internal condition of the plant. Thus it seemed possible that fruiting plants of different species might have a similar type of CO_2 exchange if flowering is the result of a common physiological condition, although different or opposite cultural environments are required to induce blossom differentiation.

The utilization of CO_2 by nonblossoming (long-day) and blossoming (short-day) poinsettia plants (fig. 3) is shown in figures 9 and 10. Particular attention is called to figures 11 to 13. When the photoperiod was reversed and the fruiting situation altered (figs. 3, 4,) the CO_2 exchange curve was altered, or, more correctly, the respiration change preceded the appearance of a change in external growth character. Figure 12 shows that different branches of a single plant have different CO_2 exchange types corresponding to the fruiting situation (fig. 4, *A*). Again when the flowering condition of the branches on a single plant was reversed (fig. 4, *B*) the CO_2 exchange rhythm was found to be reversed (fig. 13). Figure 14 records the difference in CO_2 exchange type between long-day plants some of which had become reproductive when pot bound. Fruitfulness rather than environment was the factor associated with the more irregular respiration.

As figure 15 shows, celery which was made reproductive by chilling (*6*) had an irregular CO_2 exchange type. Two later attempts to secure a reproductive condition by chilling failed. Apparently the plants were too old when chilled for this treatment to induce seedstalks. Figure 16 shows the CO_2 exchange of celery plants which were made reproductive by a short-day treatment.

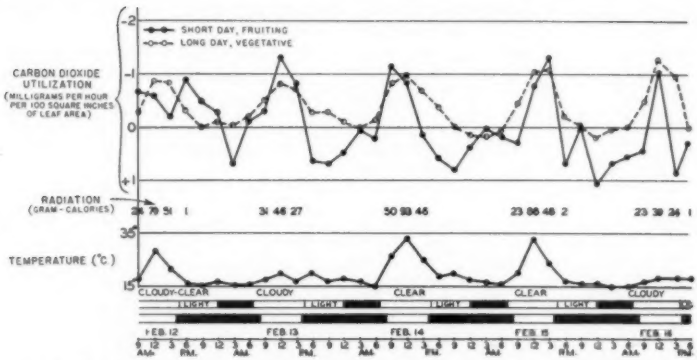

FIGURE 9.—CO_2 utilization curves of long-day, nonfruiting and short-day fruiting poinsettia plants (fig. 3). This species is one of the few under observation which appears to take up CO_2 throughout the period of added illumination. A regular curve is characteristic of the vegetative condition and an irregular curve of the reproductive condition. Values are of approximately one-tenth aliquots and show milligrams of CO_2 per hour per 100 square inches of leaf area. Minus values show CO_2 extracted from the air, and plus values CO_2 added to the air.

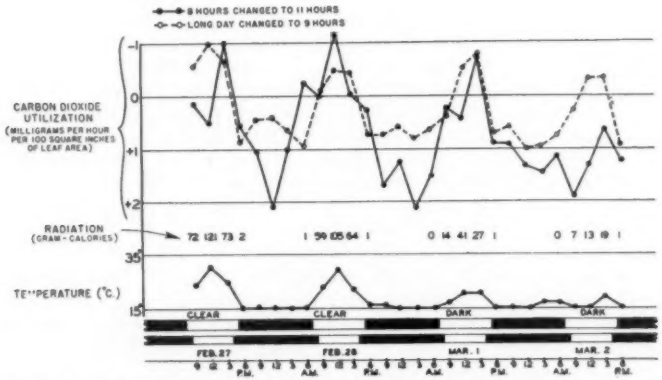

FIGURE 10.—CO_2 utilization by poinsettia plants. Short-day plants in 11-hour day and previously long-day plants changed to a 9-hour day. Immediate irregularity of the CO_2 curve is apparent, when compared to figure 9.

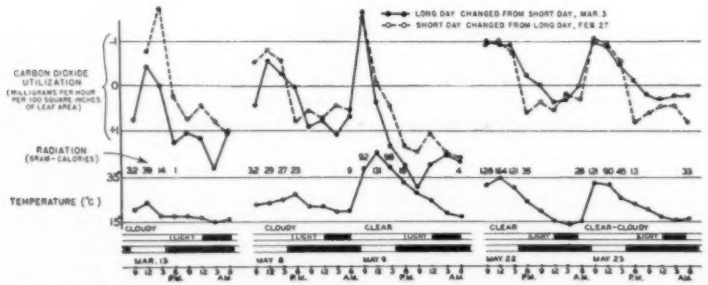

FIGURE 11.—Reversing of the photoperiod environment of poinsettia plants gave a reversal of the CO_2 utilization type (regularity of curve) as well as of fruiting condition (fig. 3).

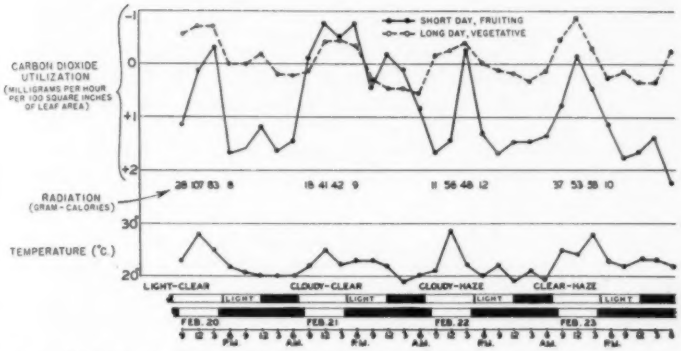

FIGURE 12.—CO_2 utilization curves of fruiting and nonfruiting branches of a single poinsettia plant (fig. 4, *A*).

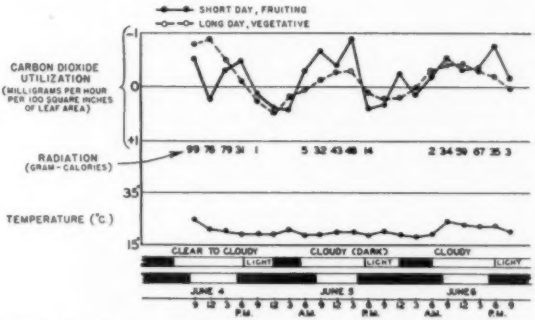

FIGURE 13.—Reversal of the photoperiod environment of the branches of single plants of poinsettia reversed the CO_2 exchange curve type and changed the reproductive condition (fig. 4, *B*).

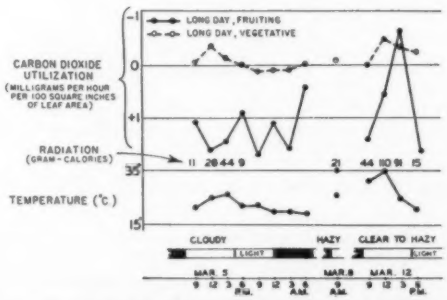

FIGURE 14.—Long-day plants of poinsettia which became reproductive in a pot-bound soil had a typically irregular CO_2 utilization curve.

Datura plants become reproductive under short-day conditions (fig. 5, *F*), but they will also blossom and set fruit if kept for a sufficient length of time under long-day conditions. (Like the tomato, the datura produces blossom primordia when growing rapidly, but such plants do not develop and set fruit.) Figure 17 shows the more irregular CO_2 exchange curve that accompanies the reproductive condition.

Klondike cosmos is a short-day plant which seems to be little affected in its blossoming by other factors than photoperiod. The

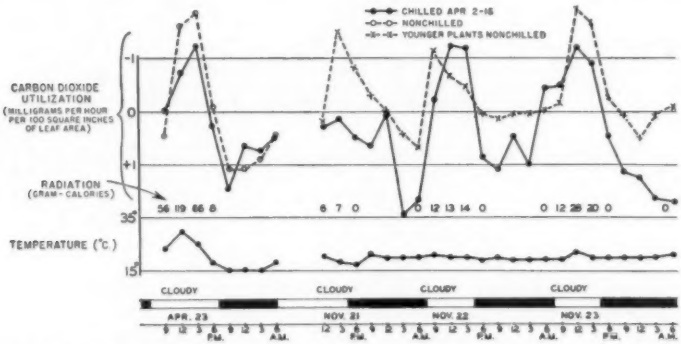

FIGURE 15.—Chilled celery plants had an irregular CO_2 exchange curve and produced seed stalks a little later (April). Old fruiting plants (November) and young vegetative plants have very different types of curves. Note the pronounced CO_2 intake in very dark weather.

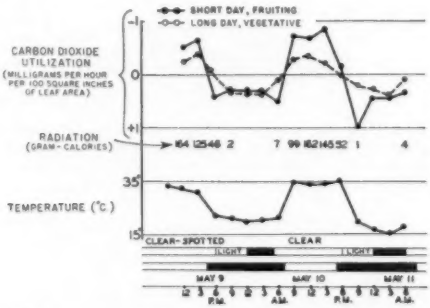

FIGURE 16.—CO_2 utilization curves of fruiting and nonfruiting celery plants grown under long- and short-day conditions.

reproductive plants had the more irregular CO_2 exchange curve (fig. 18).

The peculiar effect of photoperiod upon the flowering of ornamental sunflower plants (fig. 6) has been noted. This species seems to be less specific in its light requirements than some other plants. No suggestion is offered as to a possible correlation, but there was also less difference between the CO_2 utilization curves. The reproductive plants had the more irregular curve, however (fig. 19).

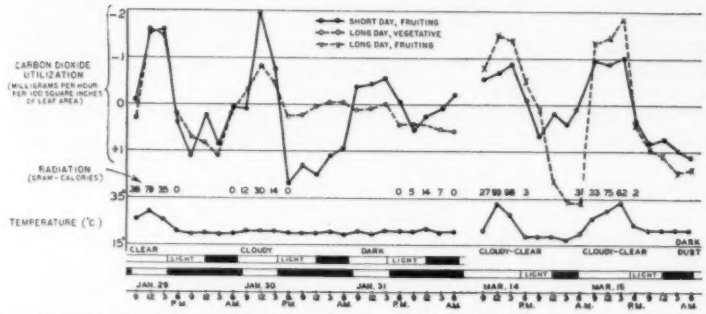

FIGURE 17.—CO_2 utilization curves of fruiting and nonfruiting datura plants (fig. 5, *E* and *F*). Dull weather reduced the CO_2 intake (Jan. 31). Long-day plants which were fruiting had irregular CO_2 curves (March).

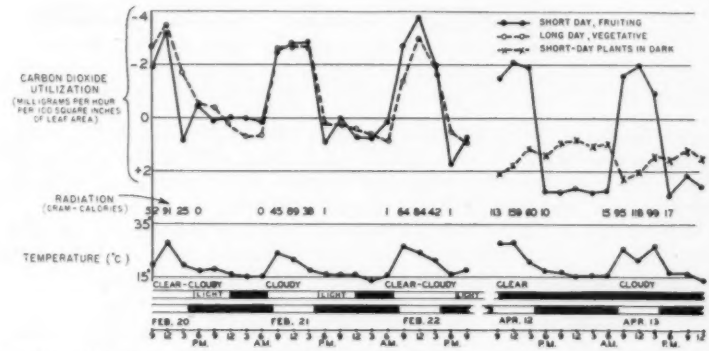

FIGURE 18.—CO_2 utilization of fruiting and nonfruiting Klondike cosmos plants. Note the cyclic character of CO_2 exchange of plants in the dark.

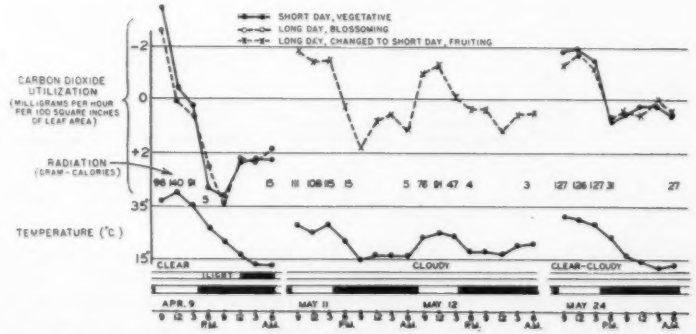

FIGURE 19.—CO_2 utilization curves of sunflower plants (*Helianthus multiflorus* Hort.). (See fig. 6.)

Vegetative spinach plants had probably the most irregular CO_2 exchange curve of any of the vegetative plants examined (fig. 20). Consequently there was less difference between the flowering and nonflowering samples. The more irregular curve was secured from the reproductive plants.

The hybrid calceolaria plants studied blossomed first in the long-day environment. The short-day plants also blossomed but at a later

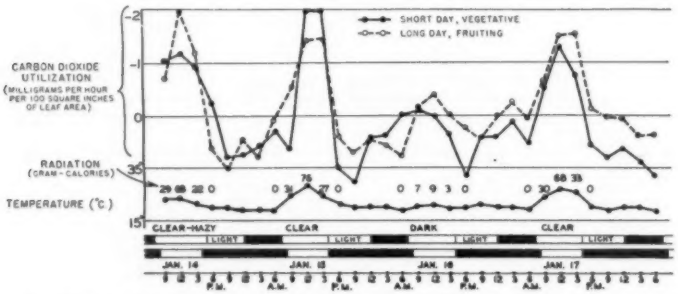

FIGURE 20.—CO_2 utilization curves of fruiting and nonfruiting spinach plants (fig. 5, *C* and *D*). Note the effect of a dark day (Jan. 16) upon CO_2 intake and the very irregular curves of the nonflowering plants.

period (fig. 21). This performance is in contrast to that of the other indeterminate plant, datura, which blossomed first in the short days. The more irregular CO_2 exchange curves were associated with the reproductive condition and not the photoperiod.

The geranium plants used blossomed first in the long-day environment (fig. 22), but belong to the indeterminate class as both long- and short-day plants blossom after different lengths of time.

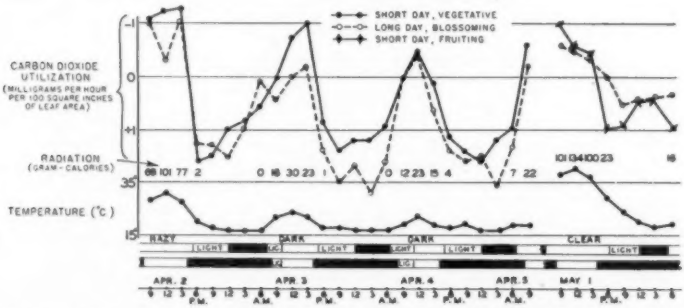

FIGURE 21.—CO_2 utilization curves of long- and short-day hybrid calceolaria plants (grown from cuttings). Long days hasten flowering; short days delay it.

Rudbeckia rootstocks must be placed under long-day conditions before any appreciable growth is made. When transferred to short-day conditions, plants which have formed flowerstalks cease elongating, a terminal rosette of leaves is formed, and the blossom buds abort unless development has been very well advanced (fig. 5, *B*). Truly vegetative stems are difficult to obtain. Figure 23 shows that

the short-day plants had a more regular respiration record than the blossoming long-day plants.

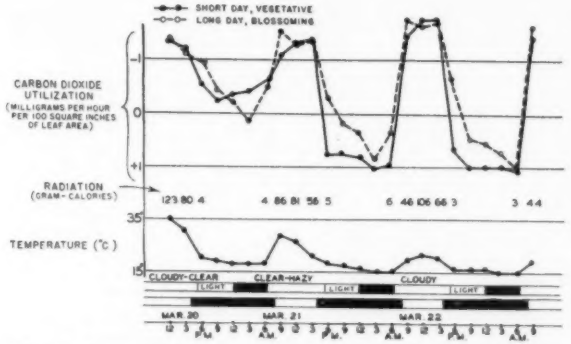

FIGURE 22.—CO_2 utilization curves of nonblossoming and bud-forming geranium plants, an indeterminate type.

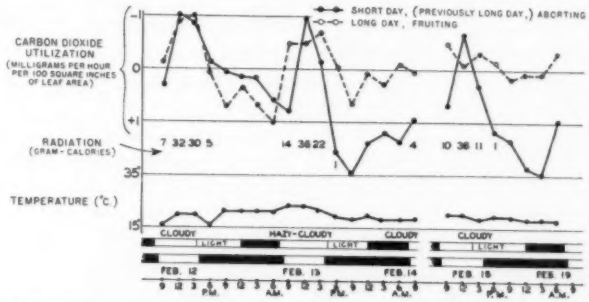

FIGURE 23.—CO_2 utilization of long- and short-day rudbeckia plants, a long-day type (fig. 5, *A* and *B*) curves.

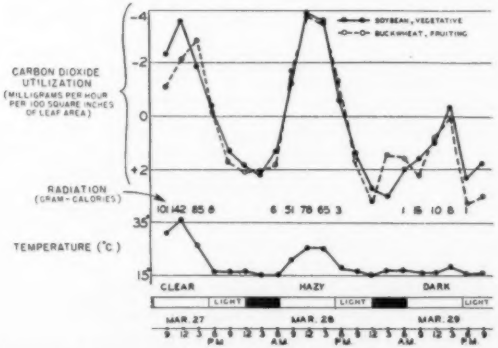

FIGURE 24.—CO_2 utilization curves of fruiting buckwheat and nonfruiting soybean plants. A dark day (Mar. 29) practically stopped CO_2 intake.

Notes on the CO_2 exchange of buckwheat, soybean, and potato (fig. 7) are shown in figures 24 and 25. The short-day plant, pigweed,

gave the data of figure 26. (Pigweed is not a short-day plant at high temperatures.)

An interesting result was secured with Maryland Mammoth tobacco (fig. 8). Plants with blossom buds gave relatively smooth

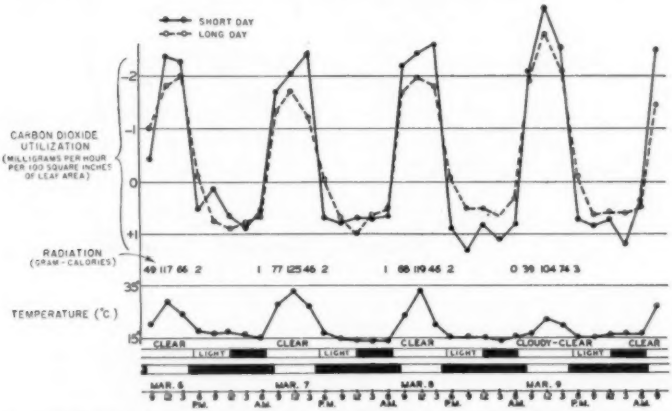

FIGURE 25.—CO_2 utilization curves of tubering (short-day) and nontubering (long-day) Triumph potato plants (fig. 7).

curves for fruiting plants (fig. 27). It was noted, however, that the buds were abscissing. The plants shortly produced vegetative shoots (fig. 8, *B*, *C*). Short-day plants grown in sand culture (low nitrogen)

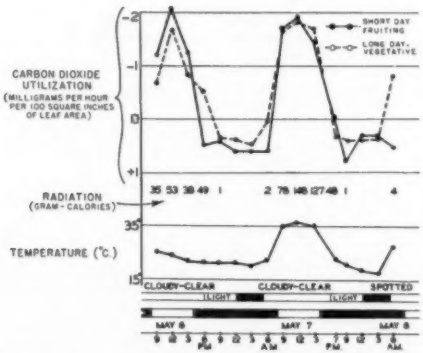

FIGURE 26.—CO_2 utilization curves of fruiting and nonfruiting pigweed (*Amaranthus*) plants.

did not lose their blossoms, but matured flowers. The CO_2 exchange curves of these plants were more irregular than those of nonflowering (blossoms abscissed) plants (fig. 28).

Ottawa flint corn had somewhat different growth characters when grown under long- and short-day conditions, but the CO_2 utilization curves of both lots were rather irregular. The plants finally became reproductive at the same time, although the short-day plants produced silk and the long-day plants produced tassels.

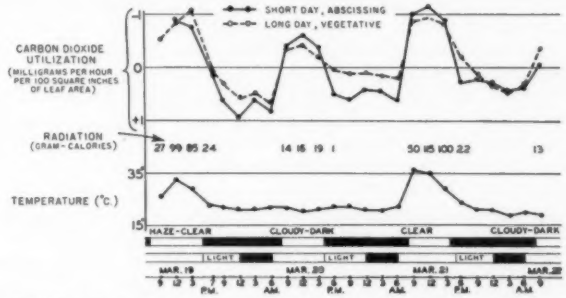

FIGURE 27.—CO_2 utilization curves of long- and short-day Maryland Mammoth tobacco (fig. 8, *A*, *B*); short-day plants abscissing their blossoms (becoming vegetative).

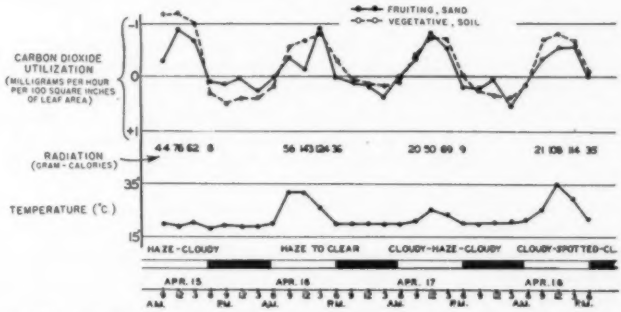

FIGURE 28.—CO_2 utilization curves of fruiting and vegetative (previously blossoming) Maryland Mammoth tobacco plants (fig. 8, *C*, *D*).

DISCUSSION

Some side lines of the studies upon the relation of CO_2 exchange to fruitfulness seem too significant not to be mentioned even at the risk of digressing from the main subject under discussion. Two of these are the influence of light intensity and the length of the illumination period upon CO_2 intake.

The failure of artificial illumination to appreciably alter the diurnal CO_2 exchange curve at the same time that it entirely altered the reproductive condition raises the question whether photoperiodism is not due to photochemical processes other than photosynthesis. Photoperiod responses took place under light conditions which did not clearly change the form of the CO_2 exchange curves.

Added illumination beyond the normal daylight period appears to have little effect upon the CO_2 exchange curves except with a very few of the species or varieties observed. In fact, many plants do not appear to carry on photosynthesis for more than a part of the daylight period. The failure to utilize the total period of light for CO_2 intake appears in some plants, such as the apple, to be due to stomatal closing in the early afternoon. The measurements of CO_2 here recorded very obviously do not show the use that the plant makes of CO_2 but indicate only the balance between the CO_2 given off during respiration and that taken up for photosynthesis.

Next to the relation of CO_2 exchange to reproductive condition, the most important deduction from the present data concerns the effect of the internal environment upon CO_2 utilization. The data from the apple (tables 1 to 6) show that the effect of the external environment upon CO_2 uptake is not consistent, but is conditioned upon the make-up of the plant resulting from its previous history. For example, the effect of girdling the tree or of varying the nitrogen nutrient upon CO_2 uptake was found to depend upon the stock used or the length of the light period under which the trees were grown. The fact that different plant species have different types of CO_2 exchange curves and that fruiting and nonfruiting plants of the same variety use CO_2 at different rates may be considered as evidence of the influence of internal environment.

While it is clear from figures 9–28 that blossoming plants have a more irregular CO_2 curve than nonblossoming plants, the question is still unanswered whether the very clear relation between the irregularity of CO_2 exchange and blossoming condition means that the respiration rhythm is directly correlated with fruitfulness. It seems very probable that CO_2 exchange is affected by the anatomical development that takes place and that it is related to reproduction merely because a correlation exists between anatomical condition and fruitfulness. In cases where there was relatively little difference between the CO_2 utilization curves of fruiting and nonfruiting plants, such as those of spinach and tobacco, there also appears to be relatively little difference in the general anatomical characters of the two groups (7).

Since all of the reproductive plants have an irregular CO_2 exchange curve and all have a more irregular curve than the truly nonreproductive plants of the same variety or species, in spite of different environments being necessary to induce flowering, it may be concluded that reproduction accompanies a common physiological condition. It would greatly advance the study of fruitfulness should it be determined that blossoming is a common physiological phenomenon in plants.

SUMMARY

The internal condition of the plant resulting from its past environment has a marked effect upon its CO_2 utilization.

The form of the CO_2 exchange curves is apparently affected in part by stomatal movement; for example, a reduction in CO_2 intake by apple leaves in the early afternoon followed stomatal closing.

Apparently other photochemical effects than photosynthesis may be responsible for the phenomena of photoperiodism. At least photoperiod responses are secured under light conditions which do not give CO_2 intake.

The irregular form of the CO_2 exchange curve which accompanies the reproductive stage may be directly correlated with anatomical characters, and so related to the fruiting condition.

The fact that the type of the CO_2 intake and respiration curves is correlated with the reproductive state of the plant is taken to be an indication that a common physiological condition is present in all of the reproductive plants under observation.

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GROWTH, REPRODUCTION, FEEDING, AND WING DEVELOPMENT OF THE MEALY PLUM APHID IN RELATION TO CLIMATIC FACTORS [1]

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INTRODUCTION

The following study was conducted entirely with those forms of *Hyalopterus pruni* (Geoff.) which occur on plums, the primary host. These forms are (1) *fundatrices*—apterous, parthenogenetic, viviparous females which hatch from overwintered eggs; (2) *fundatrigeniae*—apterous, parthenogenetic, viviparous females which are borne from

FIGURE 1.—Cloth-cellophane cages used to cage *Hyalopterus pruni* on plum trees in a lath house. Eight-inch pots were used.

fundatrices or other fundatrigeniae; and (3) *migrantes*—alate, parthenogenetic, viviparous females which, after reaching maturity, fly to the secondary hosts.

METHODS

The aphids used in the following studies were obtained from eggs collected in Placer and San Joaquin Counties, Calif. The eggs were kept out of doors, at San Jose. All of the fundatrices which hatched on any one day (average 32.7 per day) were placed on a potted plum tree (Agen or Grand Duke) and covered with a cloth cage, as shown in figure 1. The fundatrices which hatched during the first part of the hatching period were called first-hatched fundatrices and used as

[1] Received for publication Apr. 28, 1936; issued April 1937.

the progenitors of the first-born, or maximum, series of generations. The fundatrices which hatched during the latter part of the hatching period were used as the progenitors of the last-born, or minimum, series of generations.

As soon as the fundatrices in a cage matured, all were removed but one, and her young were daily removed and counted. All of the young born on a given day by the first-hatched fundatrices, were transferred to a new tree, in a new cage, until 10 such cages were established. These are referred to as the first-generation, first-born series, fundatrigeniae. Similarly, as soon as these aphids matured

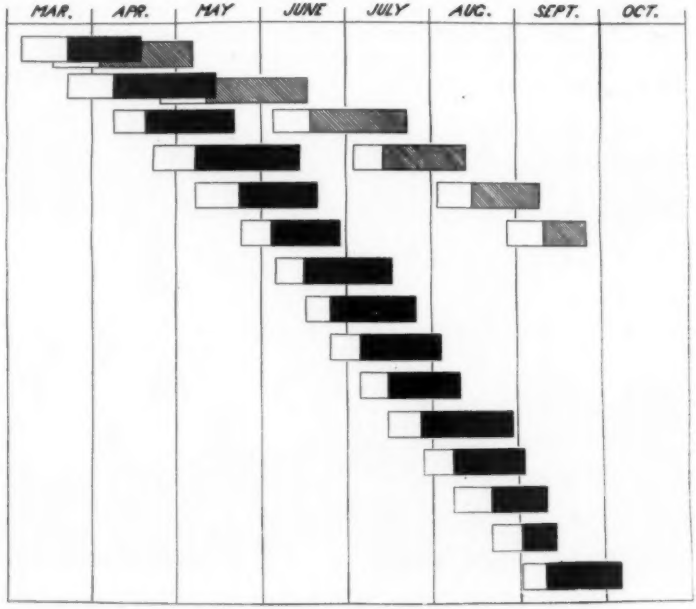

FIGURE 2.—First- and last-born series of generations of fundatrigeniae. White indicates developmental period; black, reproductive period of first-born series; cross-hatched, reproductive period of last-born series. The two rectangles at the top represent fundatrices.

and bore young, their young were transferred to new cages. After the necessary 10 cages had been established the remainder of the young produced by any set of females of the first-born series, were destroyed.

No young were established from the last-hatched fundatrices until these females showed signs of senescence, at which time all of the young born each day were put in a cage, and this practice was followed until 10 cages had been established. These are referred to as the first-generation, last-born series, fundatrigeniae. When these aphids matured and produced young, the young were counted and destroyed daily until the females showed signs of senescence, at which time the second-generation, last-born series fundatrigeniae were established.

This process, which was commenced on March 5 was continued until October 4, at which time 15 generations of the first-born series

and 6 generations of the last-born series had been completed. Their relation to one another is shown graphically in figure 2.

The surface of the soil in each pot was covered by a disk of green blotting paper which fitted closely about the base of the tree. Each cage was removed daily, at the same hour, and all exuviae mounted on slides in euparal. These were later examined microscopically and the length of an antenna and a metatibia of each skin determined. These dimensions served admirably to distinguish the molts from one another until midsummer, as indicated in figure 3. Later the combined data of length of antennae and time of molting were necessary to distinguish the molts.

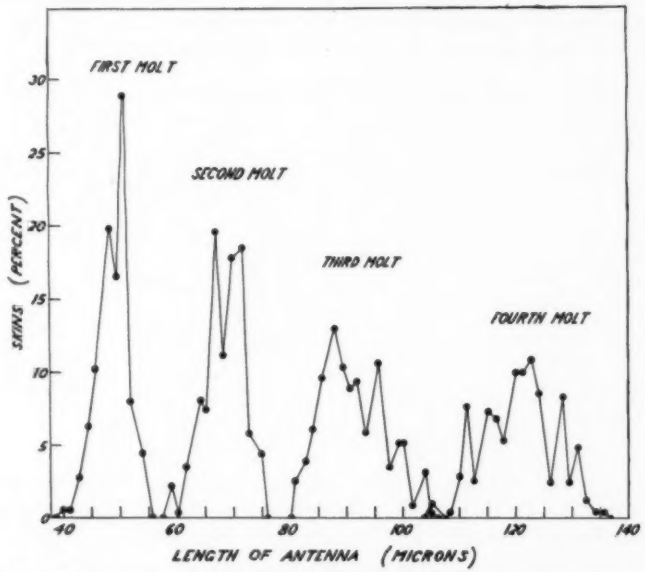

FIGURE 3.—Relation of antennal length in the several instars of fundatrigeniae. Note the increasing variation in each instar, as indicated by the range in antennal length.

From the collected and identified skins, the length of each instar in each cage for each generation was computed by the formula:

$$I=\frac{\Sigma(D\times M)}{\Sigma(M_1)}-X$$

where I is the length of the instar in days; D, the number of the day, numbered consecutively after birth; M, the number of molts of a given type occurring on the particular day D; Σ, summation; M_1 all of the skins of the type dealt with, for example third molt; and X the number of the day of the preceding molt, or day of birth.

A maximum-and-minimum thermometer was kept in one of the cages and read daily. A second thermometer of this type was kept in the shade in the lath house. A thermograph was located in a standard kiosk about 50 yards from the cages. The temperature

in the cages varied from −1° to +2.5° C. from the temperature of the air. The mean daily temperature used herein is half of the sum of the maximum and the minimum recorded by the thermometer inside of one of the cages.

Humidity records were obtained from a continuous recorder in a well-ventilated shelter about 50 yards from the cages. The mean daily humidity in the following study is one-twelfth of the sum of readings every second hour.

RELATION OF DEVELOPMENT TO TEMPERATURE AND HUMIDITY

The length of the developmental period was taken from the time of birth until the first young were born. The mean period of development shown by all the cages of each generation, together with the mean daily temperatures and humidities, is shown in table 1.

TABLE 1.—*Developmental period as related to temperature and humidity*

Series and generation	Mean developmental period	Mean temperature	Mean relative humidity	Series and generation	Mean developmental period	Mean temperature	Mean relative humidity
	Days	*° C.*	*Percent*	First-born fundatrigeniae—Continued.	*Days*	*° C.*	*Percent*
First-hatched fundatrices	19.0	14.4	.....	Eleventh	10.3	19.8	72.8
First-born fundatrigeniae:				Twelfth	11.2	18.0	73.8
First	15.8	13.7	62.6	Thirteenth	11.9	18.1	70.4
Second	13.8	14.4	60.3	Fourteenth	11.8	19.6	66.7
Third	15.0	15.2	69.4	Last-hatched fundatrices	16.4	14.4	.....
Fourth	11.4	17.3	68.0	Last-born fundatrigeniae:			
Fifth	11.8	17.0	62.4	First	16.0	15.7	70.0
Sixth	11.4	17.6	60.8	Second	11.3	17.5	60.8
Seventh	10.2	19.2	.....	Third	9.6	20.9	67.8
Eighth	9.0	20.8	70.7	Fourth	9.8	19.8	73.7
Ninth	10.6	20.1	69.0	Fifth	11.8	19.6	66.7
Tenth	11.3	18.8	74.4				

The relation of the rate of development to mean temperature is shown graphically in figure 4. The regression of rate of development on temperature is shown by the straight line. This was computed from the formula

$$\overline{A}=M_A+\left(\frac{\Sigma AX-(\Sigma A)(M_x)}{\sqrt{\Sigma A^2-(\Sigma A)(M_A)}\sqrt{\Sigma X^2-(\Sigma X)(M_x)}}\right)\left(\frac{\sqrt{\Sigma A^2-(\Sigma A)(M_A)}}{\sqrt{\Sigma X^2-(\Sigma X)(M_x)}}\right)(X-M_x)$$

as presented by Wallace and Snedecor (*22*),[2] wherein A represents any percent of development per day; X, any mean temperature; M_A, mean of A; M_x, mean of X; and $\overline{A}$, estimated value of A.

In this study no account has been taken of the time elapsed at night when the temperature fell below the threshold of development. In spite of this fact, the coefficient of correlation between temperature and rate of development was 0.89. This correlation is sufficiently high to permit the calculation of the approximate developmental period of the plum aphid in any section of California from the mean

[2] Reference is made by number (italic) to Literature Cited, p. 363.

temperature. The formula is: Percent development per day=0.56 (mean temperature, °C.)−1.4.

In the calculation of this formula and in the graph (fig. 4) only fundatrigeniae were considered. It is interesting to note, however, that the mean period of development of the fundatrices was 17.7 days at a mean temperature of 14.4° C. Their development was, therefore, at the rate of 5.7 percent per day. The estimated rate of development of fundatrigeniae at this temperature is

$$0.56\times14.4-1.4=6.7 \text{ percent development per day.}$$

The fundatrices developed in the early spring, and the night temperatures, no doubt, fell below the developmental zero. This may account

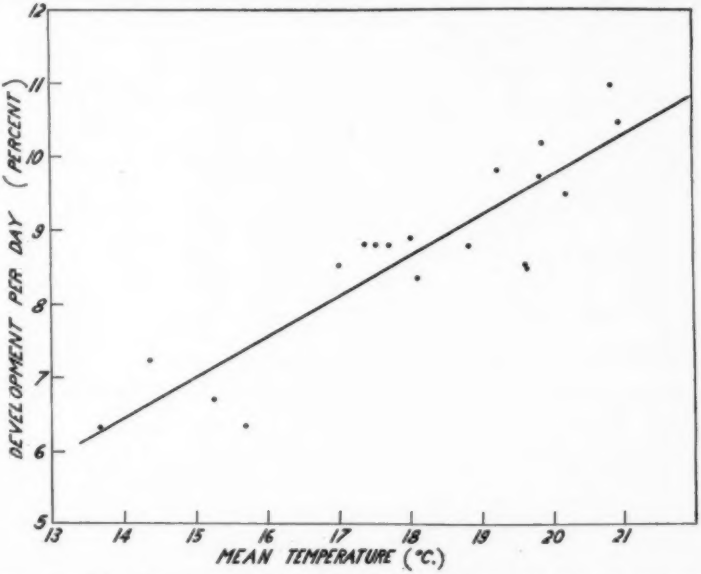

FIGURE 4.—Relation of percent development per day to mean temperature obtaining during the develop mental period.

for the discrepancy between the experimentally determined rate of development of the fundatrices and the calculated rate of development of the fundatrigeniae. It may well be that these two unlike forms respond identically to temperature.

The threshold of development, or developmental zero, indicated by these data is 2.5° C.

The role of humidity in the determination of rate of development is uncertain. It is difficult to divorce the effect of humidity from the effect of the coexistent temperatures. The coefficient of correlation between the rate of development, expressed in percent development per day, and the relative humidity, expressed in percent of saturation, is −0.30. The correlation between the temperature and relative

humidity is 0.48. The correlation between the development per day and the saturation deficiency, computed from the measured temperatures and relative humidities and expressed in grams per cubic meter, is 0.54.

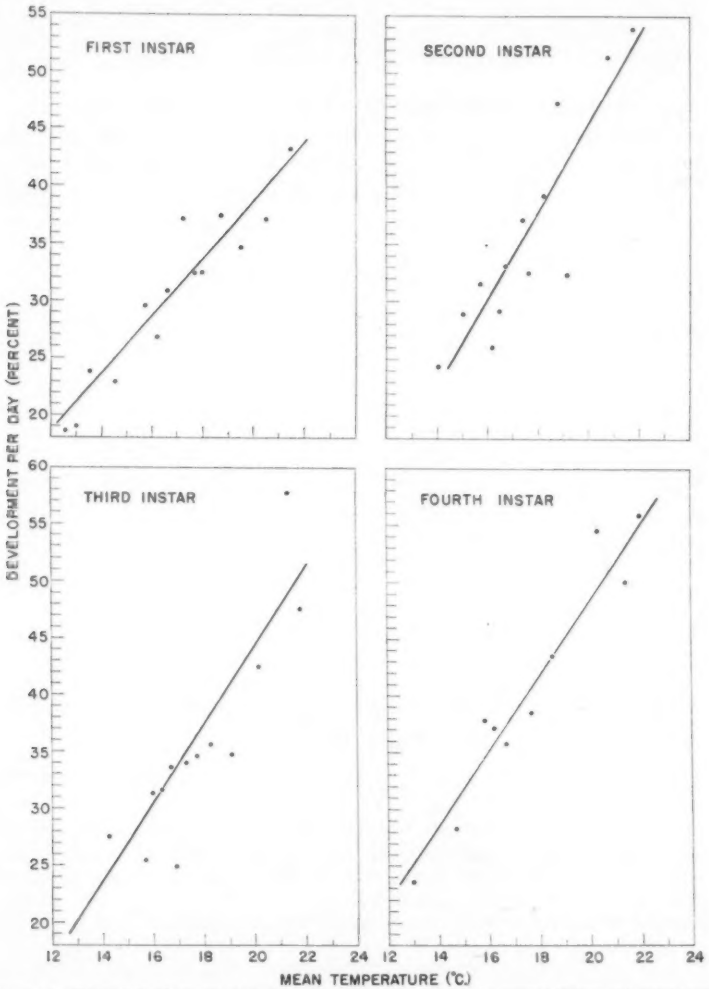

FIGURE 5.—Rate of development at various mean temperatures for the several instars of fundatrigeniae data from table 2.

The development of migrantes was slower than the development of fundatrigeniae. When a number of migrantes and fundatrigeniae, born at the same time, were reared in the same cage, the fundatrigeniae invariably reached maturity a day or two before the migrantes. In all, 118 migrantes were reared at a mean temperature of 19.5° C.

Their development from birth to completion of the fourth molt required, on an average, 12.6 days, or 7.93 percent of development per day. Fundatrigeniae, at this temperature, completed their development from birth to production of first young at the rate of 9.52 percent of development per day. The fundatrigeniae, therefore, complete their development 1.59 percent per day faster than migrantes, at a mean temperature of 19.5° C.

From the identified exuviae the length of the various instars was determined. These periods, together with the mean temperature are presented in table 2. The mathematical relations of these data are indicated in table 3 and presented graphically in figure 5.

TABLE 2.—*Rate of development of fundatrigeniae, as related to mean temperature for the 4 instars*

First instar			Second instar			Third instar			Fourth instar		
Length instar	Mean temperature	Development per day	Length instar	Mean temperature	Development per day	Length instar	Mean temperature	Development per day	Length instar	Mean temperature	Development per day
Days	*° C.*	*Percent*	*Days*	*° C.*	*Percent*	*Days*	*° C.*	*Percent*	*Days*	*° C.*	*Percent*
5.38	12.5	18.6	4.14	14.0	24.2	3.64	14.2	27.5	4.24	13.0	23.6
5.26	13.0	19.0	3.46	15.0	28.9	3.94	15.7	25.4	3.54	14.8	28.2
4.20	13.5	23.8	3.16	15.7	31.6	3.20	16.0	31.3	2.66	15.9	37.6
4.36	14.5	22.9	3.84	16.2	26.0	3.16	16.3	31.6	2.70	16.3	37.0
3.40	15.7	29.4	3.44	16.4	29.1	2.98	16.6	33.6	2.80	16.8	35.7
3.76	16.2	26.6	3.02	16.7	33.1	4.02	16.9	24.9	2.60	17.8	38.5
3.26	16.6	30.7	2.68	17.3	37.3	2.94	17.3	34.0	2.30	18.6	43.5
2.70	17.3	37.0	3.08	17.6	32.5	2.88	17.7	34.7	1.84	20.4	54.3
3.10	17.7	32.3	2.54	18.2	39.4	2.80	18.3	35.7	2.00	21.5	50.0
3.10	18.0	32.3	2.10	18.8	47.6	2.88	19.1	34.7	1.80	22.2	55.6
2.68	18.7	37.3	3.10	19.2	32.3	2.35	20.2	42.6	----------	----------	----------
2.89	19.5	34.6	1.94	20.7	51.5	1.74	21.3	57.5	----------	----------	----------
2.70	20.5	37.0	1.85	21.6	54.1	2.10	21.8	47.6	----------	----------	----------
2.30	21.5	43.5	----------	----------	----------	----------	----------	----------	----------	----------	----------

TABLE 3.—*Mathematical relations between development and mean-temperature data for the 4 instars*

Item	First instar	Second instar	Third instar	Fourth instar
Coefficient of correlation, rate of development, and temperature	0.93	0.89	0.86	0.94
Regression equation	Percent=(2.46)(° C)—10.90	Percent=(3.89)(° C.)—32.08	Percent=(3.45)(° C.)—24.89	Percent=(3.23)(° C.)—16.77
Development zero	4.43° C.	8.24° C.	7.2° C.	5.2° C.
Standard deviation, percent development per day	8.8	9.6	8.7	9.1

The average rate of regression of all four instars of the percent of development per day on the mean daily temperature is indicated by the formula

$$\text{Percent}=(3.26)(^\circ\text{ C.})-21.16$$

The average deviation of the rate of regression of each instar from the mean rate is:

Instar:	*Percent*
First	[1] 3.34
Second	.21
Third	.50
Fourth	3.88

[1] Computed by the formula

$$\frac{(R_1{}^1-R_M{}^1)+(R_1{}^2-R_M{}^2)+(R_1{}^3-R_M{}^3)\ \ldots\ldots}{N}$$

in which $R_1{}^1$ is the regression equation of the first instar at a given temperature (e. g. 12°) and $R_M{}^1$ is the regression equation of the mean rate at the same temperature. $R_1{}^2$, $R_1{}^3$, $R_1{}^4$, etc., are the regression equations of the first instar at several temperatures and $R_M{}^2$, $R_M{}^3$, $R_M{}^4$, etc., are the regression equations of the mean rate at the same temperatures. N is the number of temperatures, in this case, 11.

From these data, it may be concluded that *Hyalopterus pruni* completes four instars of approximately equal duration at any given mean temperature. Thus, at a mean temperature of 20° C. each instar will be completed in 2.27 days.

RELATION OF REPRODUCTION TO TEMPERATURE

The number of young borne each day by each female was recorded. As will be shown later, the age of the females greatly affected the rate of production of young. However, during the major portion of the reproductive period the rate of production of young bears a

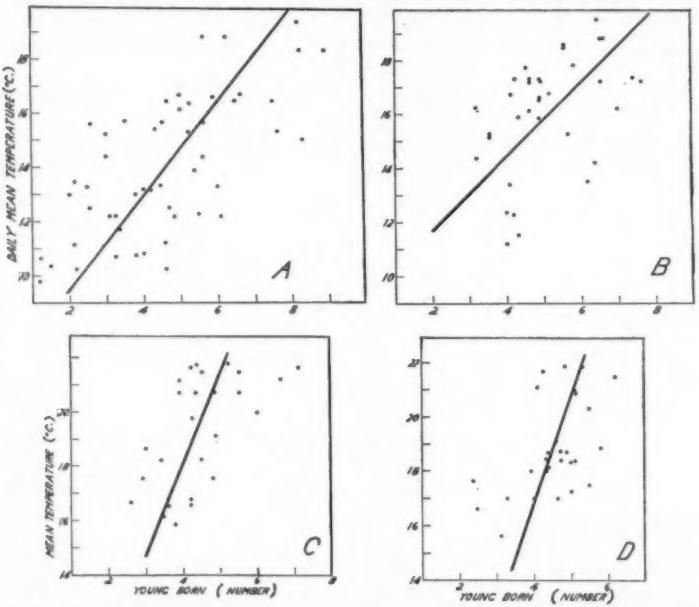

FIGURE 6.—Rate of reproduction at various mean temperatures: *A*, Fundatrices, correlation 0.67; *B*, fundatrigeniae, group 1, reproductive period, April 4 to June 4, mean temperature 16.1° C.; correlation 0.75 *C*, fundatrigeniae, group 2, reproductive period June 1 to July 12, mean temperature 19.4°; correlation 0.56; *D*, fundatrigeniae, group 3, reproductive period July 1 to September 1, mean temperature 18.9°, correlation 0.54.

definite relation to the mean temperature. Only this portion, therefore, has been used in the study of the relation of reproduction to temperature. It extends generally from the third to the twentieth or twenty-fifth day of the reproductive period. Beyond that time senescence interferes, as is shown under reproduction and age (p. 11).

When the numbers of young borne by both the first and the last-borne series of fundatrices were summarized on temperatures the following figures were obtained:

Average temperature (° C.):	*Average number of young born*
12.3	3.9
13.3	3.8
14.3	4.3
15.4	5.1
16.5	5.8
18.6	7.3
19.4	8.1

The data upon which the above summary is based are represented graphically in figure 6, *A*. The correlation between mean daily temperature and number of young born is 0.67. The mathematical regression of rate of reproduction on daily mean temperature is indicated by the formula

$$\text{Number of young born} = (0.57)(^\circ \text{C.}) - 3.44,$$

and is shown by the line in figure 6, *A*.

The 14 generations of fundatrigeniae (first- and last-born series) were similarly studied. Each generation indicated a relation between rate of reproduction and temperature, but when taken together the correlation was slight. Consequently these generations have been divided into three groups: (1) Those which bore young from April 4 to June 4, during which time the mean temperature was 16.1° C.; (2) those which bore young from June 1 to July 12, during which time the mean temperature was 19.4°; and (3) those which bore young from July 1 to September 1, during which time the mean temperature was 18.9°. The data, thus grouped, are presented as 3-day averages in table 4.

TABLE 4.—*Rate of reproduction of 3 groups of fundatrigeniae as related to temperature*

Group 1 [1]				Group 2 [2]				Group 3 [3]			
Average young born	Mean temperature	Average young born	Mean temperature	Average young born	Mean temperature	Average young born	Mean temperature	Average young born	Mean temperature	Average young born	Mean temperature
Number	° *C.*	*Number*	° *C.*	*Number*	° *C.*	*Number*	° *C.*	*Number*	° *C.*	*Number*	° *C.*
4.0	11.2	6.0	19.5	2.9	17.5	3.9	21.1	5.1	20.9	4.8	18.4
7.0	16.2	3.3	16.2	3.6	16.6	2.6	16.6	4.4	18.1	4.7	17.0
7.4	17.3	4.4	15.9	4.9	19.1	4.5	18.2	5.1	18.4	5.1	21.0
6.2	13.6	4.7	16.1	5.5	21.5	6.6	21.1	4.0	17.0	4.4	18.0
6.4	14.2	4.1	16.8	4.2	19.8	4.8	20.8	4.1	21.0	4.6	19.1
4.3	11.5	5.6	17.3	4.1	21.5	4.4	20.8	3.9	18.0	3.3	17.0
4.2	12.3	7.5	18.9	3.8	15.9	4.2	21.7	4.8	21.9	4.3	18.5
3.3	14.4	6.3	17.3	3.5	16.1	3.9	20.7	5.3	21.9	5.8	18.9
4.2	17.3	5.5	16.6	4.2	16.8	5.5	20.7	4.9	18.7	5.5	17.5
4.1	13.4	4.4	15.1	6.0	20.0	7.1	21.7	4.4	18.7	6.2	21.5
4.0	12.4	4.3	18.7	4.8	17.5	5.2	21.9	5.0	18.4	4.3	21.8
7.7	17.1	4.9	17.3	4.2	16.6	4.4	21.9	2.4	17.6	2.5	16.7
5.8	15.2	6.6	18.9	3.4	18.2	3.0	18.7	5.5	20.3	4.7	18.8
6.5	17.2	4.7	17.3					5.0	17.3	3.1	15.6
4.5	17.7	4.9	16.6								
5.9	17.8	3.6	15.1								
5.2	16.8	5.6	18.7								
4.9	15.9										

[1] Mean temperature 16.1° C.; total 38 females; correlation 0.75.
[2] Mean temperature 19.4° C.; total 35 females; correlation 0.56.
[3] Mean temperature 18.9° C.; total 38 females; correlation 0.54.

The data in table 4 are represented graphically in figure 6, *B*, *C*, *D*; and the mathematical regression of rate of reproduction on mean temperature is shown. The rate of reproduction of group 1 is approximately the same as that of the fundatrices, whereas the rates of groups 2 and 3 differ but are similar to one another. The rate of reproduction of these two latter groups is seen to be much slower at high temperatures than the first two. Thus, at 19° C. groups 2 and 3 produced about 4.5 young per day, whereas group 1 and the fundatrices produced about 7.3 young per day. An acclimatization may

have occurred in the midsummer generations (groups 2 and 3) or the trees may have been less vigorous and less food may have been available to the reproducing females of these groups.

The formulas representing the rate of reproduction as indicated by daily mean temperature are

$$\text{Fundatrices, young, daily} = (0.57)(^\circ\text{ C.}) - 3.44$$
$$\text{Fundatrigeniae, group 1, young, daily} = (0.72)(^\circ\text{ C.}) - 6.4$$
$$\text{Fundatrigeniae, group 2, young, daily} = (0.29)(^\circ\text{ C.}) - 1.17$$
$$\text{Fundatrigeniae, group 3, young, daily} = (0.27)(^\circ\text{ C.}) - 0.55$$

The length of the reproductive period as well as the total number of young produced is related to the temperatures affecting reproducing females during the reproductive period. The relation of these two items to the mean temperature obtaining during the reproductive period is shown in table 5. In general, an increase in mean temperature shortened the length of the reproductive period and decreased the total young produced by each female.

TABLE 5.—*Relation of the mean temperature during the reproductive period to length of reproductive period and number of young produced*

Generation and series	Mean temperature	Maximum reproductive period	Total average young per female	Average young per day per female
	°C.	*Days*	*Number*	*Number*
First-hatched fundatrices	14.0	41	129.9	4.7
First-born, first-generation fundatrigeniae	15.0	37	165.5	4.5
Last-hatched fundatrigeniae	15.2	35	136.5	3.9
First-born, second-generation fundatrigeniae	15.8	36	131.5	3.7
First-born, third-generation fundatrigeniae	17.2	29	128.8	4.4
First-born, fourth-generation fundatrigeniae	17.8	31	132.0	4.3
First-born, fifth-generation fundatrigeniae	18.2	24	112.2	4.7
Last-born, third-generation fundatrigeniae	18.8	30	110.1	3.7
First-born, tenth-generation fundatrigeniae	18.8	27	107.1	4.0
First-born, ninth-generation fundatrigeniae	18.8	27	107.4	4.0
First-born, twelfth-generation fundatrigeniae	19.1	29	80.3	2.8
First-born, eleventh-generation fundatrigeniae	19.2	21	88.1	4.2
Last-born, second-generation fundatrigeniae	19.4	18	99.2	5.5
Last-born, fourth-generation fundatrigeniae	19.6	26	75.1	2.9
First-born, eighth-generation fundatrigeniae	19.6	27	105.5	3.9
First-born, sixth-generation fundatrigeniae	19.7	26	106.0	4.1
First-born, seventh-generation fundatrigeniae	19.8	25	116.6	4.7
Average of totals	18.0	28.8	117.3	4.1

RELATION OF REPRODUCTION TO AGE OF FEMALES

As a result of the method of establishing each cage of each generation (see Methods), every female in each generation was a different age on any one day. Thus, in a chronological arrangement of the 10 cages of a given generation, each female was 1 day older than the next; and on any one day females ranged from 1 to 10, or 9 to 18, etc., days old. Consequently, the average number of young born on any one day of the reproductive period corresponds to the average temperature of the 10-day period during which they were born. Four generations, with reproductive periods of representative mean temperatures, are given in table 6. The data presented have been smoothed by a 5-day sliding average and are represented graphically in figure 7. These curves show that, with each increase in mean temperature, the mode of the curve of reproduction occurs earlier in the reproductive period. Thus, the mode at 14° C. occurs at about the fourteenth day, whereas at 19.6° the mode occurs on the sixth

day of the reproductive period. Another point worthy of notice is that the rate of decrease in reproduction is slower at higher temperatures. It is possible that at 14° each female deposits her complement of young by approximately the twenty-second day of the reproductive period, and the rate of reproduction then falls rapidly as a result of exhaustion of the ovaries.

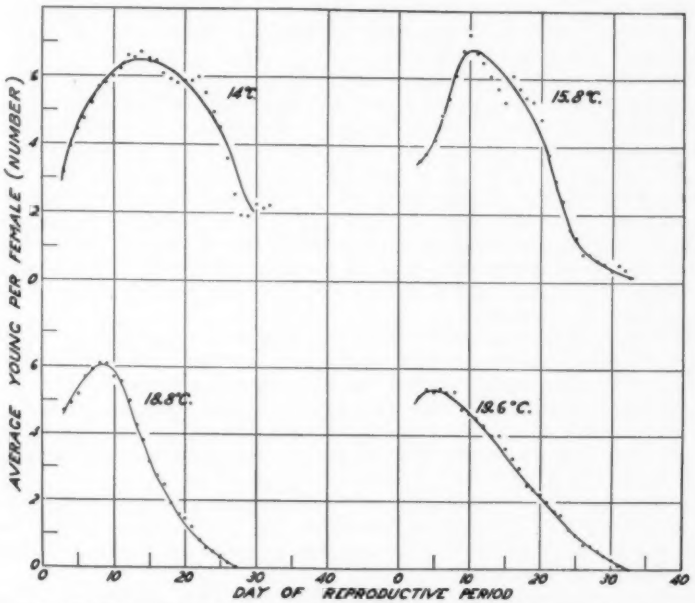

FIGURE 7.—Daily production of young throughout the reproductive periods of four generations representing several mean temperatures, as indicated; data from table 6. See text for discussion.

TABLE 6.—*Effect of age on reproduction at different mean temperatures*

Day of reproductive period	Average young per female at mean temperature of—				Day of reproductive period	Average young per female at mean temperature of—			
	14.0° C.	15.8° C.	18.8° C.	19.6° C.		14.0° C.	15.8° C.	18.8° C.	19.6° C.
	Number	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*
First	1.5	2.4	4.4	4.4	Twenty-third	6.0	2.6	1.1	2.0
Second	2.7	2.6	4.3	5.6	Twenty-fourth	6.4	.6	.6	.3
Third	3.9	3.2	4.4	5.1	Twenty-fifth	3.0	2.0	.2	1.0
Fourth	3.5	5.4	4.8	5.2	Twenty-sixth	3.2	.5	.0	.5
Fifth	4.5	4.2	5.7	4.6	Twenty-seventh	4.0	1.0	.6	1.0
Sixth	4.9	3.8	5.6	5.5	Twenty-eighth	1.5	.0	.0	.5
Seventh	5.4	4.4	5.6	5.1	Twenty-ninth	1.0	.5	.2	.5
Eighth	5.8	6.6	6.7	5.5	Thirtieth	.0	1.0	.0	.0
Ninth	5.4	8.0	5.1	4.8	Thirty-first	3.0	.0	.0	.0
Tenth	6.8	7.8	6.4	5.4	Thirty-second	6.0	1.0	.0	.0
Eleventh	5.8	7.2	6.6	3.3	Thirty-third	1.0	.0	.0	.0
Twelfth	6.2	6.8	5.5	4.8	Thirty-fourth	2.0	.0	.0	.0
Thirteenth	7.2	3.8	4.9	4.3	Thirty-fifth	2.0	.0	.0	------
Fourteenth	7.5	6.3	3.9	3.5	Thirty-sixth	8.0	.0	.0	------
Fifteenth	6.4	6.5	4.3	4.0	Thirty-seventh	4.0	.0	.0	------
Sixteenth	6.8	5.0	2.9	3.2	Thirty-eighth	1.0	.0	.0	------
Seventeenth	5.6	4.8	3.1	3.0	Thirty-ninth	5.0	.0	.0	------
Eighteenth	6.1	8.0	1.6	3.0	Fortieth	2.0	.0	.0	------
Nineteenth	5.6	4.3	1.7	1.6	Forty-first	.0	.0	.0	------
Twentieth	5.5	5.0	2.1	1.8	Forty-second	.0	.0	------	------
Twenty-first	6.4	4.3	1.0	2.5	Forty-third	.0	.0	------	------
Twenty-second	5.8	2.3	1.0	2.0					

RELATION OF RATE OF FEEDING TO TEMPERATURE

The rate of feeding was measured by the rate of deposition of excrement. Feeding charts, described below, indicated that excretion is a continuous process over considerable periods of time. The volume of excrement deposited at a regular rate exceeds the capacity of the body of the aphid, so we may conclude that digestion and feeding are likewise continuous processes synchronized to excretion. Hence measurement of the rate of excretion is in turn a relative measure of the rate of feeding.

The method of depositing excrement is unique. The tip of the abdomen is raised, a globule of transparent colorless liquid is secreted and held for an instant on the tip of the abdomen. The left posterior leg is then swung upward and outward. The drop of excrement is caught by the tip of the tibia and base of the tarsus and thrown with considerable force. From a height of 6 inches females frequently throw excrement laterally for a distance of 6 to 8 inches. The mass of each droplet and force and direction of the throw are remarkably uniform. At a distance of several inches all droplets are deposited in a space no larger than a 10-cent coin (provided the position of the aphid has not changed).

The excrement is colorless and soon evaporates, leaving no visible mark. Various methods were used to render the excrement visible. The best method involved the use of chemical pH indicators. The most efficient indicator was bromcresol green. When added to ordinary white paper this material turned pale yellow, as at pH 4.2. When drops of excrement fell on paper treated with this indicator and dried, they produced brilliant blue dots, as at pH 5.6. The blue dots contrasted markedly with the yellow background of the prepared paper. The size of the dots was remarkably uniform for each stage, and after study, the various instars could be determined from the size of the droplets.

Aphid excrement was found to reduce the blue pigment of blueprint paper, leaving a white dot. This method, however, was less satisfactory than the bromcresol-green method, since the composition of blueprint paper varies, and results from it were not dependable.

Sheets of white paper were coated with a solution of bromcresol green. When dry, they were cut into disks, which could then be mounted on clockwork and caused to revolve slowly below a feeding, excreting aphid.

Two types of records were kept. First the deposition of a single isolated specimen, in which case the angle between drops was computed into minutes and this into drops per hour and recorded, together with the mean temperature during the interval between drops. Such records are herein referred to as continuous feeding. At intervals of from 2 to 15 hours the aphids apparently ceased feeding, since excretion ceased. After a pause, usually of a few hours, excretion was recommenced. Such pauses were not computed into the continuous-feeding data.

The second type of record consisted of clock-driven charts showing the defecation of a know number of aphids. In this case, hour intervals were laid off on the disk, and the number of drops of excrement per hour per aphid recorded, together with the mean temperature. This type of record is herein termed "discontinuous feeding" since

it is presumed that some of the group are not feeding at all times. The data gathered by these methods are summarized in table 7.

TABLE 7.—*Effect of temperature on rate of feeding* [1] *as indicated by deposition of excrement*

Mean temperature (° C.)	Drops of excrement per hour per aphid for—				Mean temperature (° C.)	Drops of excrement per hour per aphid for—			
	Group of females bearing young	Isolated females bearing young	Group of third- and fourth-instar nymphs	Isolated fourth-instar nymphs		Group of females bearing young	Isolated females bearing young	Group of third- and fourth-instar nymphs	Isolated fourth-instar nymphs
	Number	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*
34.5	----------	----------	1.2	----------	19.4	0.6	----------	1.4	-------
32.8	1.0	----------	1.5	----------	17.8	.8	0.4	.9	-------
31.1	.7	----------	.9	4.0	16.1	.7	.9	.8	2.3
29.5	.9	2.7	2.2	3.8	14.4	.9	.4	1.4	1.7
27.8	.7	3.2	1.0	3.0	12.8	.5	.8	1.5	1.6
26.1	1.3	3.0	1.5	3.1	11.1	1.0	----------	1.3	1.4
24.5	1.2	2.1	1.7	3.2	9.4	----------	----------	1.1	1.6
22.8	1.2	2.0	1.4	----------	7.8	----------	----------	1.2	1.4
21.1	1.0	1.3	1.1	2.8	6.1	----------	----------	1.1	1.1

[1] Continuous for isolated females and isolated nymphs but not for the other groups.

In table 7 the columns containing data for isolated females and isolated nymphs refer to continuous feeding; the other columns refer to discontinuous feeding. By dividing each column into a higher and lower half, averaging, and computing the mean temperature, the increase in rate of feeding for a rise of 10° C. was found to be:

Continuous feeding:
- Females, producing young ---------- 1.85 drops per hour
- Fourth-instar nymphs ---------- 1.34 drops per hour

Discontinuous feeding:
- Females, producing young ---------- 0.20 drop per hour
- Fourth-instar nymphs ---------- 0.13 drop per hour

RELATION OF THE PRODUCTION OF MIGRANTES TO EPIGENETIC FACTORS

Although this section deals with the influence of epigenetic factors upon the production of migrantes, a word must be said in passing of the relation of the production of these forms to genetic factors.

Shull (*16*) while studying *Macrosiphum gei* (Koch) (*solanifolii* (*Ashmead*)) found that winged parents showed a strong tendency to produce wingless offspring (2:5), and vice versa (5:2).

Ackerman (*1*) stated that in the grain aphid, *Rhopalosiphum prunifoliae* (Fitch), winged parents are less likely than wingless parents to have winged offspring.

Mason (*12, v. 7, p. 3*) after studying *Lachnus pini* Linn. (*Cinara pini* (Linn.)) stated "external conditions and environmental changes do not effect the production of winged forms of aphids, at least not in the first generation." A carefully drawn conclusion was reached by Ewing (*7, pp. 312–313*), who wrote:

> It appears that in the case of the apple-grain aphid, and presumably in similar species of aphids, there is a type of inheritance not dependent upon the germplasm (or at least the chromosomes) but which modifies the expression of somatic char-

acters which are dependent upon the germinal elements in the following important ways: (a) through the general metabolism of the individual which in turn is affected by its environment; (b) in such a way as to produce cumulative and hold-over effects from generation to generation; (c) through cumulative effects to produce an unbalanced state of metabolism which eventually rights itself by returning towards the mean of the race. The term physiological inheritance might be applied to this parental and ancestral transmission of cumulative and oscillating metabolic inertia.

Shull (*19*) studied a green strain and a yellow strain of *Macrosiphum gei*. He established the fact that these color variations were not genetic or heritable. However, green forms produced more winged offspring than yellow forms.

Other investigators have indicated that epigenetic factors are the primary determinants of wings. Lathrop (*9*) stated that whereas *Aphis avenae* Fab. becomes entirely winged in the second generation in New York, the majority are not winged until the fourth generation in Oregon.

Comstock (*4*) reared 98 consecutive wingless generations through a period of 4 years. No winged forms appeared, except in the sixtieth generation when the aphids were allowed to become crowded.

Reinhard (*14*) reared 59 apterous generations of *Aphis gossypii* Glover under uniform conditions and no winged forms appeared.

While rearing fundatrigeniae of *Hyalopterous pruni*, as described earlier in this paper, the author kept careful records of the number of winged and wingless offspring produced. These counts for each generation, together with the mean temperature, are shown in table 8.

TABLE 8.—*Relation of the several generations of fundatrigeniae of Hyalopterous pruni to the production of migrantes*

Series and generation of parents	Offspring reared	Winged offspring	Mean temperature of reproductive period	Series and generation of parents	Offspring reared	Winged offspring	Mean temperature of reproductive period
	Number	*Percent*	° *C.*	First-born fundatrigeniae—Continued.	*Number*	*Percent*	° *C.*
First-hatched fundatrices		0.0	14.0	Tenth generation	205	12.7	18.8
First-born fundatrigeniae:				Ninth generation	963	15.9	18.8
First generation		.0	15.0	Eleventh generation	176	20.5	19.2
Second generation	103	1.9	15.8	Eighth generation	360	36.1	19.6
Third generation	181	.0	17.2	Sixth generation	540	12.6	19.7
Fourth generation	206	.0	17.8	Seventh generation	553	28.2	19.8
Fifth generation	416	12.5	18.2				

In table 8 no relation between generation and wing production can be found. The migrantes arose from parents subjected to a high mean temperature during their reproductive period, and a cumulative or increasing tendency to produce winged offspring does not appear in increasingly later generations.

From the foregoing brief review of the literature relating to direct genetic control of the production of winged offspring, it seems likely that fundatrigeniae alatae are more prone to produce apterous offspring than are fundatrigeniae apterae, provided other conditions are uniform. This is the only conclusion on genetic control which seems established. In the following study of *Hyalopterus pruni* only funda-

trigeniae apterae were used, so it may be concluded that we are dealing with a uniform group as far as genetic control of the production of migrantes is concerned.

Investigators are quite generally agreed that epigenetic factors are primarily responsible for wing determination. The factors which have been stated to influence the ratio of apterous to alate offspring are: Light, temperature, crowding, starvation, and the chemical constitution of the host plant.

The effect of light was probably first noted by Marcovitch (*10, 11*), who found that winged forms of *Aphis forbesi* Weed were produced in May on strawberry plants subjected to 7½ hours of sunlight daily, whereas these forms normally appear in November. Conversely, when a long light day was simulated, winged forms did not appear until December.

Some of the best-planned and most carefully conducted work on the problem of wing determination in aphids has been done by Shull (*17, 18*) on the influence of light on *Macrosiphum gei*. He found, among other interesting discoveries, that either continuous light or continuous darkness caused offspring to be wingless. Light exposures of 5, 8, and 12 hours daily caused offspring to be winged.

Temperature is the best-known factor in wing determination. More data have accumulated on this subject than on the others, probably because temperature is easily measured and controlled.

Ewing (*6*) found that a maximum percentage of apterous forms were produced by *Aphis avenae* at 70° F. As the temperature varied both ways, the percentage of winged forms increased.

Call (*2, p. 20*) found that at 84° to 90° F. no winged forms of *Aphis maidis* Fitch were produced, whereas at 60° to 70° large numbers of winged forms appeared.

Wadley (*21*) found an appreciably higher percentage of winged offspring in *Rhopalosiphum prunifoliae* at 62° F. than at 80°.

Ewing (*7*) found that more wingless forms of the apple grain aphid, *Rhopalosiphum prunifoliae*, were produced at 65° F. than at temperatures higher or lower.

On the other hand, some authors have failed to find a relation between temperature and wing development. Thus Clarke (*3*) reared *Macrosiphum rosae* (Linn.) at several temperatures and found no effect on wing development. Smith (*20*) found that the temperature changes at the approach of winter were not the primary cause of the production of winged forms of *Anuraphis bakeri* Cowen. Reinhard (*14*) found that temperature changes failed to produce winged forms in *Aphis gossypii* Glover.

In examining the data collected on *Hyalopterus pruni* the author has associated the percentage of winged forms developed every 4 days with the mean temperature of the 4 days preceding, since it has been shown by Shull (*17, 18*) and others that wing determination probably occurs on the second or third day preceding birth. The observations grouped in this manner are summarized in table 9.

The data in table 9 are shown graphically in figure 8. The correlation between temperature and percentage of winged offspring produced is 0.96, which may indicate a straight-line relation as shown by the line of regression in figure 8.

TABLE 9.—*Summary according to temperatures,*[1] *of percentage of winged offspring computed on 4-day intervals*

Mean temperature ° C.	Offspring reared	Winged offspring	Mean temperature ° C.	Offspring reared	Winged offspring	Mean temperature ° C.	Offspring reared	Winged offspring
	Number	*Percent*		*Number*	*Percent*		*Number*	*Percent*
14	----------	0.0	18	1,009	11.1	21	425	21.4
15	80	2.5	19	822	8.4	22	438	18.7
17	595	7.9	20	887	13.1	23	582	23.9

[1] Mean temperature of the 4 days preceding birth.

Several investigators have noted that as the aphid population on a plant becomes large, and actual crowding occurs, winged forms are produced. Thus Comstock (*4*) reared 98 wingless generations, and winged forms appeared only in the sixtieth generation, when the aphids were allowed to become crowded. Ackerman (*1*) stated that crowding increased the percentage of winged offspring from wingless females in the case of *Rhopalosiphum prunifoliae*. Reinhard (*14*) found that crowding produced winged offspring in *Aphis gossypii*. In the cages which contributed the data presented in table 9 crowding was not allowed to occur.

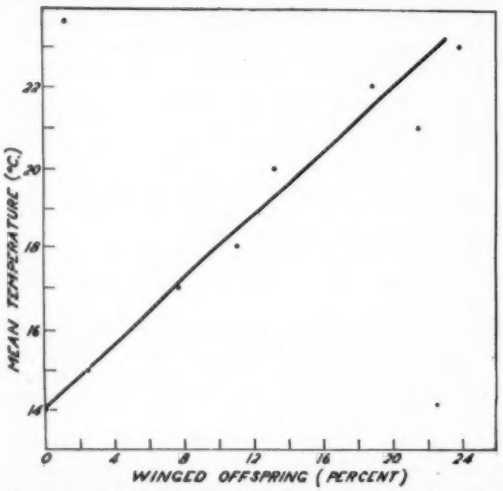

FIGURE 8.—Relation of the mean percentage of migrantes developed every 4 days, to the mean temperature of the 4 days preceding their birth; data from table 9.

Starvation, which is, no doubt, the immediate result of crowding, has been studied by removing bearing females from their hosts for a time each day. Reinhard (*14*) found that starvation increased the number of alate individuals in the progeny produced by apterous parents of *Aphis gossypii*. Shull (*17*) found that starvation increased the winged offspring from *Macrosiphum gei*. Ackerman (*1*) stated that starvation of *Rhopalosiphum prunifoliae* increased the percentage of winged offspring from wingless parents. Gregory (*8*) found that starvation of *Macrosiphum destructor* increased the percentage of winged offspring. Wadley (*21*) stated that starvation increased the percentage of winged offspring of *R. prunifoliae*.

Wilting of the host plants, which no doubt results in starvation of the aphids, has been cited by Woodworth (*23*) as productive of winged aphids in *Aphis brassicae* Linn. (*Brevicoryne brassicae* (Linn.). Smith (*20*) records similar observations on the effect of wilting of host plants upon *Anuraphis bakeri*.

Several investigators have studied the effect upon wing development of adding salt solutions to the host plant, either by placing cuttings in a solution, growing plants in modified nutrient solutions, or by watering potted plants with salt solutions. Clarke (*3*), using the "cuttings" method, found that magnesium salts produced a high percentage of winged offspring in *Macrosiphum rosae*, while sodium and potassium salts did not. Neiils (*13*) repeated Clarke's experiments, with approximately the same results. Shinji (*15*) found that magnesium salts produced wings, whereas sodium, chlorine, potassium, calcium, barium, alum, and distilled water did not. Wadley (*21*) found that magnesium sulphate, $\frac{N}{10}$, in Pfeffer's solution increased the percentage of winged offspring of *Rhopalosiphum prunifoliae* on wheat plants. Ackerman (*1*) repeated the experiments of Shinji, Clarke, and Neiils with entirely negative results, and concluded that salts were not a factor in wing determination. The most pertinent observation on this problem of the effect of salts was made by Davidson (*5*) who placed bean cuttings, supporting *Aphis rumicis* Linn., into $\frac{M}{50}$ $MgSO_4$ solutions. The aphids ceased feeding. Stains were added to the solutions and it was found that the aphids ceased feeding when the solution reached them.

The foregoing abbreviated review of literature pertaining to wing determination in aphids has been presented in an attempt to bring the various theories into harmony with one another, and to show that at the bottom of each theory may lie the same cause of wing determination, namely, the nutrition of the mother, and through her the nutrition of the embryo. Starvation by removing the females from their hosts is a direct and obvious cause. Wadley (*21*) stated that starvation completely masked the determinant effect of temperature. Crowding of the aphids and wilting of the host plant must certainly result in starvation. Salt solutions render the plant juices unsuitable for food for the aphids, again resulting in starvation, as indicated by Davidson (*5*). Light is difficult to correlate with starvation, unless it is assumed that small quantities of light are sufficient to raise the temperature of the body of the aphid.

As far as the author is aware no attempt has yet been made to determine the relation of temperature to starvation. By combining the data presented in this paper on the rate of reproduction and the rate of feeding, it is found that at higher temperatures less food is consumed per young born than at lower temperatures, and a condition of relative starvation must occur at high temperatures. The data presented in table 10 are computed from formulae determined in the sections on rate of reproduction and rate of feeding. An arbitrary starting point of 0.5 drop of excrement per hour at 11° C. was selected. As indicated in table 7, this is approximately correct. The starting point is of no consequence in this connection, however, since the factor of importance is the relative rate of increase.

Continuous feeding does not occur for any length of time; consequently, the data pertaining to discontinuous feeding represent the actual condition which obtains in nature. They have been used in computing the data in table 10, and are shown graphically in figure 9. The data on discontinuous feeding indicate that decidedly less food

per young is consumed at higher temperatures. Thus, in group 1, whereas eight drops of excrement per young were deposited at 11° C., only two drops per young were deposited at 21°, and it may be concluded that the young born at 11° obtained four times as much food as those born at 21°.

TABLE 10.—*Relation of temperature to amount of feeding per young born as indicated by deposition of excrement*

Mean temperature (° C.)	Drops of excrement per day [1]	Young born per day [2]	Drops of excrement per young born	Mean temperature (° C.)	Drops of excrement per day [1]	Young born per day [2]	Drops of excrement per young born	Mean temperature (° C.)	Drops of excrement per day [1]	Young born per day [2]	Drops of excrement per young born
	Number	*Number*	*Number*		*Number*	*Number*	*Number*		*Number*	*Number*	*Number*
11	12.0	2.0	6.0	15	13.9	3.7	3.8	19	15.8	5.4	2.9
12	12.5	2.4	5.2	16	14.4	4.1	3.5	20	16.3	5.8	2.8
13	13.0	2.8	4.6	17	14.9	4.5	3.3	21	16.8	6.2	2.7
14	13.4	3.3	4.1	18	15.4	5.0	3.1				

[1] Discontinuous feeding.
[2] Average for groups 1, 2, and 3.

In this way temperature may be translated into starvation. The data in table 9 indicate that high temperatures are productive of winged aphids.

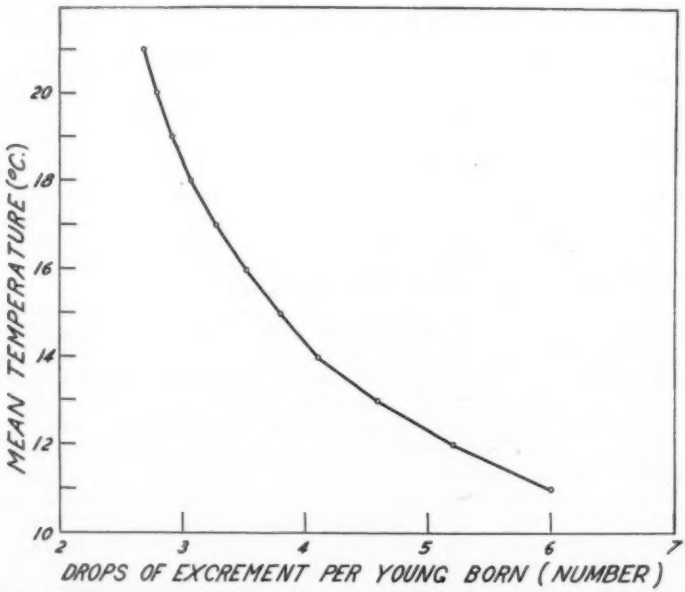

FIGURE 9.—Relation of amount of excrement deposited per young born, to various mean temperatures, showing relative starvation of young at higher temperatures; data from table 10.

When further data have accumulated on the influence of epigenetic factors on wing determination, all of the present theories may be harmonized on the basis of nutrition.

SUMMARY

A high correlation was found to exist between rate of development of the mealy plum aphid (*Hyalopterus pruni*) and daily mean temperature. Formulae for the determination of the rate of development per day at any mean temperature are presented. The four instars are of approximately equal duration at any given mean temperature. The correlation between number of young born and daily mean temperature ranged from 0.54 to 0.75 under varying conditions. Rate of reproduction was shown to be related to age of females, which in turn was influenced by temperature. The rate of feeding was related to temperature, and it was found that the rate of feeding did not increase as rapidly as the rate of reproduction, for a given increase in temperature. This results in the relative starvation of young born at higher temperatures and is offered as an explanation of wing determination.

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THE RELATION OF LIGHT TO THE DIURNAL CYCLE OF SPORULATION OF CERTAIN DOWNY MILDEWS[1]

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INTRODUCTION

The nocturnal sporulation of downy mildews, frequently observed and recorded (*1, 5, 6, 7, 8, 9*),[3] has generally been ascribed to the high humidity which is necessary for the sporulation of these fungi and which frequently prevails at night. Studies by the writer, reported herein, indicate that light and the alternation of light and darkness in the normal day may also determine the course of sporulation of four downy mildews studied.

MATERIALS

The studies reported herein deal with the downy mildew of hop, *Humulus lupulus* L., caused by *Pseudoperonospora humuli* (Miy. and Tak.) Wilson; the downy mildew of onion, *Allium cepa* L., caused by *Peronospora destructor* (Berk.) Casp.; the downy mildew of grape, *Vitis californica* Benth., caused by *Plasmopara viticola* (B. and C.) Berl. and De T.; and the downy mildew of lettuce, *Lactuca sativa* L., caused by *Bremia lactucae* E. Regel.

Because of the size of ordinary hop plants, detached living leaves were necessarily used. Five to ten days after inoculation relatively young mildewed leaves of the variety Clusters were detached near the base of the petiole, transferred to vials of water, and placed under the experimental conditions being studied. Unless otherwise mentioned in the work with onion mildew, entire onion plants of an unidentified brown variety, with 4 to 10 leaves per plant, and in 4-inch pots, were used as experimental material.

Besides studies of the direct effect of environment on sporulation of hop and onion downy mildew, many tests were made to determine the effect of pretreatment of the diseased plants on the ability of the fungus in these plants to sporulate when placed under favorable environmental conditions. Since there appears to be no word applicable to this conditioned state of the host-parasite complex, the writer will use the terms "sporulable" and "sporulability", meaning, respectively, "able to sporulate" and "capacity for sporulation." The terms "sporulable" and "sporulability" thus bear the same relation to the term "sporulate" as "germinable" and "germinability" do to the term "germinate."

[1] Received for publication Oct. 13, 1936; issued April, 1937.

[2] The writer wishes to acknowledge his indebtedness to Dr. M. W. Gardner for valuable assistance and suggestions during the course of the investigations and in the preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 373.

THE DIURNAL CYCLE OF HOP MILDEW IN THE FIELD

The sporulation of the hop mildew fungus, the dissemination of the sporangia, and the success of inoculations were successfully followed throughout the 24 hours of the day. On two occasions concurrent observations were made on weather conditions in a hop yard in Sonoma County, Calif. Sporulation was followed microscopically. At noon the sporangiophores and sporangia were washed from leaves which had been inoculated 7 days previously, and the leaf surfaces dried. At 3-hour intervals leaf samples from these leaves were folded on a slide with the lower surface of the leaf to the outside. The edge of the fold was then examined with the low- or high-power objective of the microscope. The details of development of the sporangiophores could readily be followed in this manner.

The dissemination of sporangia was ascertained by exposing glass slides as spore traps in six locations among heavily mildewed hops, changing the slides every 3 hours, and counting under the microscope the number of sporangia deposited on a given area (approximately 1 cm^2) on each slide. At each time of examination of the sporangiophores and of changing the spore-trap slides, a fresh suspension of sporangia from field plants was atomized onto the lower surface of several marked leaves. The infection from these inoculations was observed a week later.

The results of these two sets of observations, one on May 7–8, and one on May 15–16, 1935, were similar, and the observations of May 15–16 are represented in table 1. Sporangiophores were first observed at midnight, sporangiophores were fully developed and sporangia were being formed at 3 a. m., the sporangia were apparently mature at 6 a. m., and many of them had been liberated by 9 a. m. The maximum dissemination of sporangia was in the period preceding 9 a. m., numerous sporangia were caught between 9 a. m. and 12 m. and between 12 m. and 3 p. m., but few were caught at other times. Inoculations were successful throughout the day, though germination and infection may not have occurred at the time of inoculation.

TABLE 1.—*The diurnal cycle of hop mildew, May 15–16, 1935*

Time	Sky	Temperature	Relative humidity	Condition of sporangiophores	Sporangia caught in 3 hours on a total of 6 cm^2, 1 cm^2 on each of 6 slides	Leaves inoculated	Leaves infected
May 15:		° *C.*	*Percent*		*Number*	*Number*	*Number*
12 m	Sunny	20	62	Atomized off			
3 p. m.	do	24	40	None	35	25	10
6 p. m.	Clear	22	48	do	9	22	21
9 p. m.	do	11	87	do	2	23	10
12 p. m.	do	7	100	Sporangiophores forming	0	20	13
May 16:							
3 a. m.	Cloudy	7	98	Sporangiophores mostly fully formed, with immature sporangia.	1	19	14
6 a. m.	Partly cloudy.	8	93	Sporangia full-sized but not detached readily.	0	16	11
9 a. m.	Cloudy, few drops rain.	17	54	Sporangia mature and many have been detached.	240	8	2
12 m	Cloudy	18	56	Sporangia mostly detached.	87	15	6

These results emphasize the cyclic behavior of the hop mildew fungus under field conditions, but throw no light on the cause of this cycle, since sporulation may be correlated with time of day, low temperature, high humidity, or darkness.

EFFECT OF LIGHT ON THE SPORULATION OF HOP MILDEW

Heavily mildewed hop leaves excised in the morning and placed in light moist chambers in the greenhouse at favorable temperatures have consistently failed to sporulate, indicating that light or the time of day was unfavorable for the sporulation of hop mildew under these conditions.

To test the direct effect of light on sporulation, mildewed leaves were exposed to artificial light at night under conditions of temperature and humidity favorable to sporulation, and checks were maintained in darkness under similar temperature and humidity conditions. The method was to immerse a watertight preparation dish containing mildewed leaves at 100-percent relative humidity within a water bath with the leaves exposed to a 150-watt Mazda lamp at a distance of about 6 cm. A light intensity of approximately 1,000-foot candles (measured with a Weston light meter) was thus shed on the leaves. In three tests one mildewed leaf was exposed with its lower surface to the light, and another with its upper surface to the light. In all cases the sporulation was checked in the light. The temperature within the preparation dish containing the leaves, as measured with a short spirit thermometer lying with its bulb directly in the light and on the surface of one of the leaves, averaged about 21° C., that of the water bath about 19.5°, and the check leaves in darkness, where sporulation occurred consistently, were at 22°. In one other test sporulation of hop mildew was completely prevented at a light intensity of 170-foot candles from a Mazda lamp. These tests show that light intensities, a great deal less than sunlight, may check the sporulation of hop mildew, and it therefore seems possible that under natural conditions the absence or reduction of light at night may be one of the factors responsible for the nocturnal sporulation.

EFFECT OF TIME OF DAY ON THE SPORULATION OF HOP MILDEW

Every 3 hours from 12 m. to 12 p. m., May 16, 1936, mildewed leaves were taken from field plants, their sporophores washed away, and the leaf surfaces dried. One set of leaves for each time of sampling was placed in a moist chamber in a bright but shaded location, and another set of leaves was placed in darkness. These leaves were examined at intervals for spore formation. Sporophore initials were first observed at 9 p. m., May 16, on leaves placed in darkened moist chambers at noon. At 12 p. m. these leaves had spores (sporangia) about one-half the full size. At 12 p. m. all mildewed leaves, including those on field plants, had sporophore initials, and only those placed in dark moist chambers at 12 m. and 3 p. m. were significantly more advanced than the others. At 4:20 a. m., May 17, all leaves had dark but not fully mature spores. This test clearly shows that the diurnal cycle of sporulability is inherent in the host-parasite complex, and not directly dependent on the environment. Were it not for this cycle, the leaves placed in dark moist chambers at 12 m. should have

sporulated about 9 hours before those placed in moist chambers at 9 p. m.

Mildew-infected leaves from greenhouse plants held at a humidity sufficiently low to prevent sporulation of the fungus were placed in dark moist chambers at different times during the day. On four days, May 12, 13, 15, and 18, 1936, hop mildew sporulated only slightly or not at all within 12 hours on infected leaves placed in moist chambers in the morning (7 a. m. to 8 a. m.), but sporulated luxuriantly on leaves placed in moist chambers in the evening (4 p. m. to 10 p. m.). These tests indicate a fairly definite diurnal cycle as due to preconditioned causes—causes which acted on the host-fungus complex before it was placed in the constant environment.

THE DIURNAL CYCLE OF ONION MILDEW

Apparently because of its higher humidity requirement for sporulation, onion mildew sporulates less frequently under field conditions than hop mildew, and for this reason it was not found convenient to study the diurnal cycle of the pathogen under field conditions in 1936. However, the appearance overnight of onion mildew sporangia on leaves which showed no sporulation the previous evening has been observed several times in the field. In the greenhouse mildew infected plants placed in lighted moist chambers at favorable temperatures in the morning have consistently failed to sporulate or have sporulated very sparsely during the daylight period, indicating as with hop mildew that light or time of day was unfavorable for sporulation under these conditions.

The writer does not agree with Cook (*2*) that free moisture on the leaves is necessary for the sporulation of onion mildew, and has consistently secured vigorous sporulation of infected potted plants with dry leaves when these are placed in moist chambers at suitable temperatures. After hot days in the greenhouse onion mildew frequently sporulates poorly or not at all, but such failures have been rare and are not involved in the tests reported here.

Tests of the direct effect of light on the sporulation of onion mildew have not given as conclusive results as with hop mildew. In four out of five tests the mildew in detached onion leaves in dark moist chambers at 22° C. sporulated much more heavily overnight than the mildew in comparable detached leaves exposed in moist chambers at about 21° to approximately 1,000 foot-candles of light; but in one test heavier sporulation was observed on the leaves in the light.

EFFECT OF TIME OF DAY ON THE SPORULATION OF ONION MILDEW

On April 14, 1936, two infected potted onion plants were placed in a darkened moist chamber in the greenhouse every 4 hours, the first at 8 a. m. Simultaneously two similar plants were placed in an undarkened moist chamber outdoors. Of the plants placed in the dark moist chamber, sporulation occurred within 12 hours on those plants placed in the chamber at 8 a. m., 4 p. m., 8 p. m., and 12 p. m. No sporulation occurred on plants placed in dark moist chambers at 4 a. m., and the sporulation on the plants of 8 a. m., 12 m., and 4 p. m. was very weak in comparison with that of the plants of 8 p. m. and 12 p. m. Sporulation occurred most promptly on plants placed in

moist chambers at 8 p. m. On these plants well-developed sporangiophores were apparent 4 hours after the plants were placed in the moist chamber. Similar results were secured in the undarkened moist chamber outdoors, except that sporulation was more vigorous here on plants placed in the chamber at 8 a. m., 12 m., and 4 p. m. than it was in the darkened greenhouse chamber already mentioned.

In the second test on May 7, 1936, two greenhouse plants, of a group inoculated April 26, were placed every 4 hours in a darkened moist chamber at 13° C. In this test the light, temperature, and humidity conditions in the moist chamber were constant. Sporulation occurred only on the plants placed in the chambers at 4 p. m., 8 p. m., and 12 p. m., and no sporulation occurred on the plants placed in the moist chamber at 4 a. m., 8 a. m., and 12 m. In four other tests sporulation was much more vigorous on plants placed in these constant-environment chambers in the afternoon and evening (4 p. m. to 8 p. m.) than on similar plants placed in the moist chambers in the early morning (4 a. m. to 8 a. m.) of the same day.

The humidity must be continuously high, not only for the formation of the sporangiophores, but also for the maturation of the sporangia. Infected plants on which the sporangia were about half-grown were removed from the greenhouse moist chamber at 3 a. m., April 24, and exposed to the dryer greenhouse atmosphere. The half-grown spores did not mature.

CAUSE OF THE DIURNAL CYCLES OF HOP AND ONION DOWNY MILDEW

The previous tests have shown that the downy mildews of hop and onion sporulate most luxuriantly in darkness, and that on infected plants grown in the greenhouse and placed under favorable conditions for sporulation, the fungus will sporulate only, or most profusely, at night. These facts indicate that some process occurring during daylight is a natural precursor of sporulation. Since obligate parasites depend on their living hosts for their food supply, and since carbohydrates and certain other organic materials are synthesized by the host during daylight and presumably partially changed or translocated at night, the downy mildews studied may depend on these labile organic materials for their nocturnal sporulation. Tests to establish this point have been only partially successful.

If labile carbohydrates are a limiting factor for the sporulation of onion mildew, it might be expected that sporulation in the low phase of the cycle (4 a. m. to 8 a. m.) could be brought about by supplying the infected leaves with sugar solution. However, injection of 5-percent sucrose or 5-percent dextrose into the internal cavity of onion leaves at 6 a. m., or 6 p. m., had no effect on the sporulation within 12 hours when the plants were placed in a moist chamber. Placing the lower part of a detached infected leaf in 5-percent sucrose or 5-percent glucose also had no effect on sporulation.

To test the possible relation of photosynthesis to sporulation, infected hop leaves, detached in the early morning, were sealed in two moist chambers, one containing 20 percent of potassium hydroxide to absorb carbon dioxide and the other containing a fermenting glucose solution to increase the carbon dioxide content of the chamber. Both chambers were exposed to light (outdoor shade in one test, and

1,000 foot-candles from a Mazda lamp in two tests) at about 21° C. from 6 a. m. to 8 p. m., and then placed overnight in dark moist chambers at 22°. The fungus in check leaves direct from greenhouse plants to moist chambers at 8 p. m., as well as the fungus in the leaves subjected to a carbon dioxide-enriched or a carbon dioxide-deficient atmosphere, sporulated luxuriantly in three independent tests; but check leaves from a plant in a dry dark chamber at 13° for 24 hours (8 p. m. to 8 p. m.) showed no sporulation (one test only). These tests confirmed previous indications as to the necessity of light in the diurnal cycle but did not indicate that the effect of light was due to photosynthesis. (The carbon dioxide content of the experimental chambers was not measured.)

SPORULABILITY INDUCED BY EXPOSURE TO LIGHT

Infected greenhouse plants were placed in a dark room at 13° C. and 49 percent relative humidity. Thereafter every 12 hours one onion plant and one hop leaf were exposed to approximately 800 foot-candles of light from a Mazda lamp at about 20° C. and high but uncontrolled humidity for about 12 hours before being placed in dark moist chambers (at 13° for onions and 22° for hops). At these same 12-hour periods one check hop leaf and one check onion plant were transferred direct from the dry dark room at 13° to moist chambers. The results, given in table 2, show that infected plants did not sporulate immediately after several hours in darkness, but that they regained sporulability when exposed to artificial light immediately previous to the incubation in the moist chamber.

TABLE 2.—*The effect of exposure to light and darkness on the sporulability of onion and hop mildew, May 26 to June 1, 1936*

Length of time in dark at 13° C., 49-percent relative humidity (hours)	Period of exposure to light after previous exposure in dark chamber	Relative sporulation[1] after 12 hours in dark at 100-percent relative humidity—		Length of time in dark at 13° C., 49-percent relative humidity (hours)	Period of exposure to light after previous exposure in dark chamber	Relative sporulation[1] after 12 hours in dark at 100-percent relative humidity—	
		On detached hop leaves at 22° C.[2]	On onion plants at 13° C.[3]			On detached hop leaves at 22° C.[2]	On onion plants at 13° C.[3]
	Hours				*Hours*		
0	0	----------	[4] 0	36	0	0	[4] 0
12	0	[5] 8	[4] 0	36	12	5	10
12	12	5	10	48	0	Trace	[4] 0
24	0	0	[4] 0	48	12	----------	1
24	12	5	10				

[1] Relative sporulation is indicated on a scale of 0 to 10; 0 indicates no sporulation, 10 indicates maximum sporulation in this test.

[2] The hop plant from which this series of leaves was taken was transferred from the greenhouse to the dark room at 8 p. m.; the leaves were detached immediately before being exposed to light, or before being placed in the humid chamber, if there was no light treatment.

[3] The onion plants of this series were transferred from the greenhouse to the dark room at 6 a. m.

[4] At the conclusion of this test these plants were removed to the greenhouse at 8 a. m., June 1, and placed in moist chambers at 8 p. m., June 1. At 8 a. m., June 2, all had sporulated heavily.

[5] According to the explanation of sporulability advanced in this paper this leaf should have failed to sporulate.

It was thought that by holding infected plants under certain conditions, the physiologic processes might be so checked that the plants would be held in a state of metabolic balance similar to that which existed when they were placed under the test conditions. For example, it seemed possible that infected plants in which the mildew

was capable of sporulation would retain that condition for a considerable period when stored at 0° C. Starting at 8 p. m., June 1, infected greenhouse onions were placed in darkness at a range of temperatures and at humidities sufficiently low to prevent sporulation. At 12 hours and 24 hours later, two plants from each temperature were placed in dark moist chambers at 13°. On check plants transferred at 8 p. m., June 1, from the greenhouse to dark moist chambers at 13°, the fungus sporulated luxuriantly. On plants held for 12 or 24 hours at 0°, 5°, 13°, and 25°, no sporulation occurred within 2 days when the plants were placed in dark moist chambers at 13°. However, after these plants were exposed to greenhouse light for 12 hours (8 a. m. to 8 p. m., June 4) they all sporulated luxuriantly in the succeeding 12-hour period (8 p. m., June 4, to 8 a. m., June 5) in greenhouse moist chambers.

EFFECT OF LIGHT ON THE SPORULATION OF GRAPE DOWNY MILDEW

Leaves of wild grape infected with downy mildew were collected at 4 p. m., June 3, 1936. During the daylight of June 4, these leaves with their petioles in vials of water were exposed to the light of the greenhouse. At 8 p. m., June 4, three heavily infected leaves were placed at 22° C. in a darkened moist chamber, and three similar leaves were placed in a moist chamber exposed to approximately 800 foot-candles of light at about 22°. At 6 a. m., June 5, the leaves in the dark chamber had sporulated luxuriantly, but none of those in the light had sporulated. The leaves subjected to light at night were placed in dark moist chambers at 22° at 6 a. m., June 5. At 9 p. m., June 5, these three leaves had sporulated luxuriantly. This behavior of grape mildew is similar to that observed by the writer in the experiments with hop mildew.

EFFECT OF LIGHT ON THE SPORULATION OF LETTUCE DOWNY MILDEW

Leaves of lettuce infected with downy mildew were collected at 3 p. m., June 5. At 9 p. m., June 5, three leaf pieces with 12 infection spots, from which the sporophores were removed, were placed in dark moist chambers at 22° C. At 8 a. m., June 6, all these lesions were sporulating. At 9 p. m., June 5, three leaf pieces similar to the above, with 13 infection spots, were placed in moist chambers at about 22° and 800 foot-candles of light. At 8 a. m., June 6, 1 of these 13 infection spots was sporulating. All leaf pieces were then placed in the dark at 22°. At 7 p. m., a total of three, and at 8 a. m., June 7, a total of six lesions were sporulating. This test, though not as conclusive as others, supports the general observation of the direct inhibitory effect of light on the sporulation of the downy mildews studied.

DISCUSSION

Although nocturnal sporulation of certain downy mildews has been recognized for some time, and although darkness is the most conspicuous feature of the night environment, a necessary relation of light and the alternation of light and darkness to the sporulation of downy mildews does not seem to have been adequately recognized. Weston (*8*) believed that dew at night was of primary importance in

determining the nocturnal sporulation of certain *Sclerospora* species, but suggested (*8, p. 782*) that, "More precise physiologic study will probably show * * * that the relationship is more subtle and complex than this would imply." Melhus et al. (*4*) indicated that neither light nor time of day was a limiting factor in the sporulation of *Sclerospora*. Hiura (*3*) also found that sporulation of *Sclerospora* would occur during the day if temperature and humidity were favorable. However, Arens (*1*) observed that hop mildew did not sporulate well in daylight, and Oltarzhevskiĭ (*6*) observed that sporulation of grape mildew was delayed in daylight. The findings of Arens (*1*) and Oltarzhevskiĭ (*6*) are in accord with those of the writer, but the findings of Weston (*8*), Melhus et al. (*4*), and Hiura (*3*), since they are concerned with a distinctly different parasite, may not be closely applicable to the present work.

The writer's conception of the diurnal cycles of sporulability of hop and onion mildews is that, owing to the alternation of light and darkness in the normal day, infected leaves gradually pass through two phases every 24 hours. There is one phase during the early morning when sporulation will not occur or will occur only slowly and sparsely, and a second phase in the afternoon and evening when sporulation will occur quickly and luxuriantly if the plants are placed in a favorable environment. The cycle described and studied is mainly one of sporulability and not of sporulation, especially with onions, for once the fungus has sporulated luxuriantly in a given leaf it will not normally sporulate as luxuriantly in the following and succeeding sporulation periods (data not presented here). The direct inhibitory effect of light on sporulation appears to be somewhat independent of the diurnal cycle of sporulability but should contribute to the fixity of nocturnal sporulation under natural conditions.

Although light was the principal environmental factor successfully manipulated in these tests and shown to be important for the sporulation of the downy mildews studied, the writer believes that a more basic cause of sporulability is the metabolic state of the host and parasite. This metabolic state would be in large part determined by the conditions of exposure to light.

The writer does not wish to minimize the importance of humidity for the sporulation of these downy mildews. A rather high humidity is essential for the sporulation of hop mildew and onion mildew, and is generally a limiting factor in the development of these diseases under natural conditions. Favorable temperatures are also necessary, though the temperature range is quite wide and is probably rarely a limiting factor under natural conditions.

No evidence to indicate that either light intensity or the length of the light and dark periods of the day can be a limiting factor in the sporulation of hop mildew or onion mildew under field conditions has been secured, but the studies reported herein indicate that the alternation of light and darkness is necessary for the normal nocturnal sporulation.

SUMMARY AND CONCLUSIONS

The course of sporulation of hop downy mildew was followed microscopically under field conditions. During May 1935 emergence of the sporangiophores through the stomata was first observed at 12 p. m.; branching of the sporangiophores was complete and small sporangia had formed at 3 a. m.; sporangia were about full size at 6 a. m.; and

sporangia were mature and readily liberated from the sporangiophores at 9 a. m. Sporangia were caught in greatest numbers on spore-trap slides at 9 a. m. and in decreasing numbers throughout the day. Very few were caught at night.

Sporulation of the downy mildews of hop, onion, grape, and lettuce was inhibited by artificial light at night.

Hop and onion mildew sporulated most luxuriously when infected leaves or plants were placed in darkened moist chambers in the late afternoon and evening, and poorly or not at all when the leaves or plants were placed in darkened moist chambers in the early morning.

Hop and onion mildew lost their ability to sporulate when infected leaves were held in darkness at low humidity and different temperatures for 12 hours or more, but recovered their ability to sporulate when these leaves were exposed for 12 hours to natural or artificial light.

It is concluded that the diurnal cycle of sporulability exhibited by hop and onion mildew is basically dependent on the alternation of light and darkness in the normal day, but in leaves known to possess a high sporulability, the actual nocturnal sporulation is directly dependent on the darkness and high humidity frequently coincident at night.

The diurnal cycle of sporulability of onion mildew was not disturbed by filling the central leaf cavity of infected leaves with 5-percent sucrose or 5-percent dextrose, or by placing cut onion leaves with their bases in 5-percent sucrose or 5-percent dextrose.

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INFLUENCE OF STRIPE RUST ON GROWTH, WATER ECONOMY, AND YIELD OF WHEAT AND BARLEY [1]

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INTRODUCTION

The importance of stripe rust (*Puccinia glumarum* (Schmidt) Eriks. and Henn.) in cereal crop production in the United States has never been accurately determined. Many investigators consider stripe rust of minor importance at present but are fully cognizant of its potential possibilities as a limiting factor in growing wheat and barley. It is well known that there are times when it takes first rank among the most destructive plant pathogens in Europe.

Other than the work of Humphrey, Hungerford, and Johnson (*5, p. 213*) [3] and of Hungerford,[4] nothing of a definite nature appears to have been done to determine the effect of stripe rust on the yield of its three cereal hosts, i. e., wheat, barley, and rye. The experiments here described were planned with a view to determining the effect of the rust on yield, plant growth, and water economy in wheat and barley.

REVIEW OF LITERATURE

Much has been written about the devastations of stripe rust, but so far as the writer is aware no actual experiments have been performed to determine the specific loss caused by this disease. Humphrey and Cromwell (*4*) reported a severe outbreak of stripe rust in Argentina in 1929 which caused considerable damage. They attributed this outbreak to the fact that there had been a complete change of wheat varieties from those grown in previous years, together with ideal weather conditions for the best growth and spread of the rust. They suggested the possibility of spores of this rust being brought into the country on plant material in shipments of wheat received from European sources.

Tu (*11*) states that stripe rust is very destructive in southern China, and according to Biffen (*1*) it is undoubtedly the most important species of cereal rust in England. Other investigators, Rivera and Corneli (*10*) and Marchionatto (*8*) in Italy, and Hecke (*3*) in Germany, give somewhat the same picture for their respective countries. Vestergaard (*12*) has also pointed out that stripe rust can and does cause appreciable damage in Sweden.

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[3] Reference is made by number (italic) to Literature Cited, p. 384.

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The literature reveals no record of any study having been made of the effect of stripe rust on the water economy of the host plant. Several studies of this kind have been made with stem rust (*13*) and leaf rust of wheat and crown rust of oats. A comprehensive review of the literature relating to the effect of leaf rust on the yield, growth, and water economy of wheat has been presented by Johnston and Miller (*6, 7*). Reference will be made later to their own contributions.

Murphy (*9*) found that crown rust on oats greatly decreased the yield and greatly increased the amount of water used per unit of dry matter. The decrease in yield was much greater than has been recorded for similar studies with leaf rust of wheat and likewise greater than for those reported herein for stripe rust.

MATERIAL AND METHODS

The experiments reported herein were conducted in the greenhouse at Moscow, Idaho. In 1933–34 the spring wheat varieties Chogat (C. I.[5] 6244) (susceptible) and Garnet (C. I. 8181) (resistant), and in 1934–35 the spring barley varieties Pannier (C. I. 1330) (susceptible) and Khanaka (C. I. 743) (resistant) were used. Both Chogat wheat and Pannier barley are completely susceptible in all growth stages to physiologic race 19, the race used in these experiments and commonly found in the vicinity of Moscow. Garnet wheat and Khanaka barley are highly resistant to this race, chlorotic areas occurring only under optimum environmental conditions.

The methods followed in growing and caring for the wheat and barley were approximately the same as those employed by Johnston and Miller (*7*) in a similar study on leaf rust (*Puccinia triticina* Eriks.). The plants were grown in 1-gallon glazed stone jars filled with a mixture of 2 parts Palouse silt loam, 1 part sand, and 1 part well-rotted manure that had been thoroughly mixed and sifted through a ⅜-inch mesh screen. The jars were provided with sand- and gravel-filled cores, extending nearly to the bottom, to facilitate watering, and covered with a layer of paraffin to reduce the loss of water directly from the soil. Thirty-two jars were used for each variety of each crop, and additional jars, unplanted but otherwise handled in the same manner, were used to determine the amount of water lost through the apparatus itself.

At the beginning of the 1933–34 experiment, all plants were subjected daily to 8 hours of artificial light, in addition to the ordinary daylight, and later to 10 hours. The additional light was supplied by eight 200-watt bulbs placed 3 feet above the growing plants and so adjusted as to make it possible to raise them as the plants matured. In 1935 the experiment was begun in late winter and additional light was not necessary. The temperature in the greenhouse varied from 58° to 62° F. during the winter months, but it sometimes went as high as 95° on warm spring days.

[5] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

The plants of each variety were divided into six groups of five jars each, two jars being held in reserve to replace any that might be lost during the season. Successive groups were first inoculated in the one-leaf stage, the three-leaf stage, the jointing stage, the boot stage, and during anthesis. One group, used as a check, was not inoculated. Those plants that were first inoculated in the one-leaf and three-leaf stages were inoculated by making a spore suspension and then applying the spores to the plant parts by means of a small scalpel. Immediately after inoculation the plants were placed in a moist chamber for 36 hours. Plants after passing the three-leaf stage were inoculated, in a large inoculation chamber, by being sprayed with tap water and dusted with urediospores. They remained in the inoculation chamber 36 hours. Following the initial inoculation each group was reinoculated at 13-day intervals until the plants were mature. The jars were kept at constant weight by being watered with tap water as necessary, and the amount of water so used was recorded.

When ripe, the plants were measured for height, the heads and tillers were counted, and the heads were measured for length. The initial heading date and date of maturity were recorded for each jar. The culms were cut off at the soil level and the roots were washed free of soil. The heads were threshed, the number of kernels per head was recorded, and the chaff was added to the straw. The roots and all above-ground vegetative parts were air-dried at room temperature for 15 days. The results for the 1933–34 experiment are reported on the basis of air-dry material. In 1935, the harvested material was kept for 18 days in an airtight container in which were suspended several sticks of glacial phosphoric acid, a powerful drying agent. All determinations were made on material dried by these methods. The yields and other data for each class of plant material and each rust period represent the total for five jars containing six plants each.

EXPERIMENTAL RESULTS

EFFECT OF STRIPE RUST ON PLANT GROWTH AND YIELD

Table 1 shows in detail the yield of grain, straw, and roots; total number of heads and kernels; number of kernels per gram; time required for heading and ripening; and the amount of water used by each group of plants of each variety after inoculation with stripe rust. The effect on the size of the heads and kernels is illustrated in figures 1 to 3.

It is at once apparent that stripe rust adversely affected the yield and the growth and greatly increased the amount of water used per unit of dry matter (straw and grain) both of the resistant and the susceptible varieties. The length of the growing period of the host plant also was increased. The effect seems to be related to the period during which the host was invaded rather than to the amount of rust that developed, since the latter was nearly constant for all inoculated lots.

TABLE 1.—*Yield, growth characters, and water requirement of a susceptible and a resistant variety each of wheat and of barley, inoculated with stripe rust at different growth stages, in the greenhouse at Moscow, Idaho*

[Each test was made on a group of 30 plants]

Variety and growth stage when inoculated	Rust infection	Total heads	Total kernels	Kernels per gram	Total tillers	Height of plant	Length of head	Period from—			Total weight of—				Total water used	Water used in production of—	
								Planting to first heading	Planting to maturity	First heading to maturity	Straw	Grain	Roots	Dry matter		1 g grain	1 g dry matter
Wheat:						*Centimeters*	*Centimeters*										
Chogat (susceptible):	*Percent*	*Number*	*Number*	*Number*	*Number*			*Days*	*Days*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Kilograms*	*Grams*	*Grams*
Seedling	85.0	32	960	70	221	83.5	4.6	129	189	60	166.1	16.4	16.0	198.5	191.0	11,646	962
3-leaf	80.0	38	1,064	68	210	88.6	5.1	127	189	62	188.5	18.8	23.0	230.3	192.0	10,213	834
Jointing	80.0	43	1,247	62	200	91.1	5.6	117	176	59	186.0	19.2	31.5	236.7	190.2	9,906	804
Boot	75.0	61	2,440	62	186	101.6	7.6	111	168	57	262.4	41.6	93.0	397.0	206.0	4,952	519
Anthesis	80.0	59	1,880	61	180	114.3	7.1	110	167	57	199.8	32.4	106.4	338.6	189.0	5,833	558
Control (noninoculated)	0	58	2,842	51	183	116.8	7.6	111	166	55	272.1	47.0	128.6	447.7	205.0	4,362	458
Garnet (resistant):																	
Seedling	2.0	56	728	34	110	106.7	5.8	83	161	78	166.2	23.2	33.2	222.6	83.0	3,578	373
3-leaf	2.0	61	854	31	110	110.5	5.8	82	152	70	182.9	25.6	31.9	240.4	81.0	3,164	337
Jointing	2.0	52	936	31	105	113.9	6.4	71	144	73	200.0	28.9	41.1	270.0	80.4	2,782	298
Boot	2.0	56	1,008	31	105	120.9	6.4	79	148	69	205.9	30.7	42.1	278.7	83.2	2,710	299
Anthesis	2.0	51	1,020	31	102	121.4	6.4	78	145	67	200.0	32.4	43.2	275.6	82.0	2,531	298
Control (noninoculated)	0	46	1,058	30	101	121.4	6.4	78	145	67	194.5	33.6	44.0	272.1	80.1	2,384	294
Barley:																	
Pannier (susceptible):																	
Seedling	80.0	102	1,752	30	139	85.2	3.8	85	129	44	116.9	58.4	12.8	188.1	193.0	3,305	1,026
3-leaf	80.0	101	2,291	31	142	94.0	3.8	84	128	44	119.6	73.9	11.5	205.0	195.0	2,639	951
Jointing	73.0	118	2,936	29	137	99.8	4.1	81	126	45	132.2	101.6	17.8	251.6	179.0	1,762	711
Boot	65.0	114	3,216	27	132	105.7	4.1	78	121	43	139.7	122.8	22.0	284.5	189.0	1,539	664
Anthesis	65.0	112	3,309	24	125	108.5	4.3	77	120	43	142.3	146.2	46.8	335.3	182.2	1,246	543
Control (noninoculated)	0	114	3,350	21	127	110.4	4.7	77	120	43	149.3	164.3	53.1	366.7	199.0	1,211	543
Khanaka (resistant):																	
Seedling	2.0	117	4,233	29	145	109.5	4.3	85	130	45	157.0	146.3	27.3	330.6	126.0	861	381
3-leaf	2.0	112	4,103	29	155	107.7	4.3	83	129	46	161.0	141.5	25.7	328.2	116.3	822	354
Jointing	3.0	119	3,958	29	141	110.3	4.6	83	130	47	155.4	136.5	23.0	314.9	115.0	842	365
Boot	1.5	121	4,595	27	145	115.3	4.8	82	129	47	184.2	170.2	26.0	380.4	129.3	760	340
Anthesis	1.5	126	4,928	28	143	113.1	5.1	81	128	47	161.5	176.0	34.2	371.7	126.0	716	339
Control (noninoculated)	0	119	4,220	27	138	114.3	5.1	81	126	45	180.3	156.3	36.0	372.6	127.4	815	342

The percentage reduction (or increase in a few cases) in the yield of total dry matter (grain, straw, and roots) is shown in table 2, and similar data for the number of heads and kernels, the number of kernels per gram, the height of the plants, length of heads, number of tillers, and number of days to heading and to maturity are also given. As would be expected, the effect is most marked for the inoculations in the early stages of growth and becomes progressively less as the date of first inoculation approaches maturity of the plant. Never-

FIGURE 1.—Head development in two wheat varieties, (*A*) Chogat (susceptible) and (*B*) Garnet (resistant), inoculated with stripe rust at the following stages: *a*, Noninoculated control; *b*, seedling; *c*, three-leaf; *d*, jointing; *e*, booting; and *f*, anthesis; and reinoculated at 13-day intervals until mature.

theless, inoculations as late as anthesis had a marked effect on the growth and development of the various plant parts and especially on the weight of roots and the yield of grain. The effect of inoculating the susceptible wheat in the boot stage was, in general, less than would be expected on the basis of earlier and later inoculations. This undoubtedly was due to failure to secure infection at the time as a result of excessive sunshine and high temperatures. Infection did take place with subsequent inoculations.

TABLE 2.—*Percentage increase or decrease in yield, growth characters, and water requirement of a susceptible and a resistant variety each of wheat and of barley, inoculated with stripe rust at different growth stages in the greenhouse at Moscow, Idaho*

[Percentage differences are based on corresponding control values (table 1)]

Variety and growth stage when inoculated	Total heads	Total kernels	Kernels per gram	Tillers	Height of plant	Length of head	Period from—			Total weight of—				Total water used	Water used in production of—	
							Planting to first heading	Planting to maturity	First heading to maturity	Straw	Grain	Roots	Dry matter		1 g grain	1 g dry matter
Wheat:																
Chogat (susceptible):	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Seedling	−44.8	−66.2	+37.3	+20.8	−28.5	−39.5	+16.2	+13.9	+9.1	−39.0	−65.1	−87.6	−55.7	−6.8	+167.0	+110.0
3-leaf	−34.5	−62.6	+33.3	+14.8	−24.1	−32.9	+14.4	+13.9	+12.7	−30.7	−60.0	−82.1	−48.6	−6.3	+134.1	+82.1
Jointing	−25.9	−56.1	+21.6	+9.3	−22.0	−26.3	+5.4	+6.0	+7.3	−31.6	−59.1	−75.5	−47.1	−7.2	+127.1	+75.5
Boot	+5.2	−14.1	+21.6	+1.6	−13.0	0	0	+1.2	+3.6	−3.6	−11.5	−27.7	−11.3	+.5	+13.5	+13.3
Anthesis	+1.7	−33.8	+19.6	−1.6	−2.1	−6.6	−.9	+.6	+3.6	−26.6	−31.1	−17.3	−24.4	−7.8	+33.7	+21.8
Garnet (resistant):																
Seedling	+21.7	−31.2	+13.3	+8.9	−12.1	−9.4	+6.4	+11.0	+16.4	−14.6	−31.0	−24.5	−18.2	+3.6	+50.1	+26.9
3-leaf	+32.6	−19.3	+3.3	+8.9	−9.0	−9.4	+5.1	+4.8	+4.5	−6.0	−23.8	−27.5	−11.7	+1.1	+32.7	+14.6
Jointing	+13.0	−11.5	+3.3	+4.0	−6.2	0	−9.0	−.7	+9.0	+2.8	−14.0	−6.6	−.8	+.4	+16.7	+1.4
Boot	+21.7	−4.7	+3.3	+4.0	−.4	0	+1.3	+2.1	+3.0	+5.9	−8.6	−4.3	+2.4	+3.9	+13.7	+1.7
Anthesis	+10.9	−3.6	+3.3	+1.0	0	0	0	0	0	+2.8	−3.6	−1.8	+1.3	+2.4	+6.2	+1.4
Barley:																
Pannier (susceptible):																
Seedling	−10.5	−47.7	+42.9	+9.4	−22.8	−19.1	+10.4	+7.5	+2.3	−21.7	−64.5	−75.9	−48.7	−3.0	+172.9	+89.0
3-leaf	−11.4	−31.6	+47.6	+11.8	−14.9	−19.1	+9.1	+6.7	+2.3	−19.9	−55.0	−78.3	−44.1	−2.0	+117.9	+75.1
Jointing	+3.5	−12.4	+38.1	+7.9	−9.6	−12.8	+5.2	+5.0	+4.7	−11.5	−38.2	−66.5	−31.4	−10.1	+45.5	+30.9
Boot	0	−4.0	+28.6	+3.9	−4.3	−12.8	+1.3	+.8	0	−6.4	−25.3	−58.6	−22.4	−5.0	+27.1	+22.3
Anthesis	−1.8	−1.2	+14.3	−1.6	−1.7	−8.5	0	0	0	−4.7	−11.0	−11.9	−8.6	−8.4	+2.9	0
Khanaka (resistant):																
Seedling	−1.7	+.3	+7.4	+5.1	−4.2	−15.7	+4.9	+3.2	0	−12.9	−6.4	−24.2	−11.3	−1.1	+5.6	+11.4
3-leaf	−5.9	−2.8	+7.4	+12.3	−5.8	−15.7	+2.5	+2.4	+2.2	−10.7	−9.5	−28.6	−11.9	−8.7	+.9	+3.5
Jointing	0	−6.2	+7.4	+2.2	−3.5	−9.8	+2.5	+3.2	+4.4	−13.8	−12.7	−36.1	−15.5	−9.7	+3.3	+6.7
Boot	+1.7	+8.9	0	+5.1	+.9	−5.9	+1.2	+2.4	+4.4	+2.2	+8.9	−27.8	+2.1	+1.5	−6.7	−.6
Anthesis	+5.9	+16.8	+3.7	+3.6	+1.0	0	0	+1.6	+4.4	−10.4	+12.6	−5.0	−.2	−1.1	−12.1	−.9

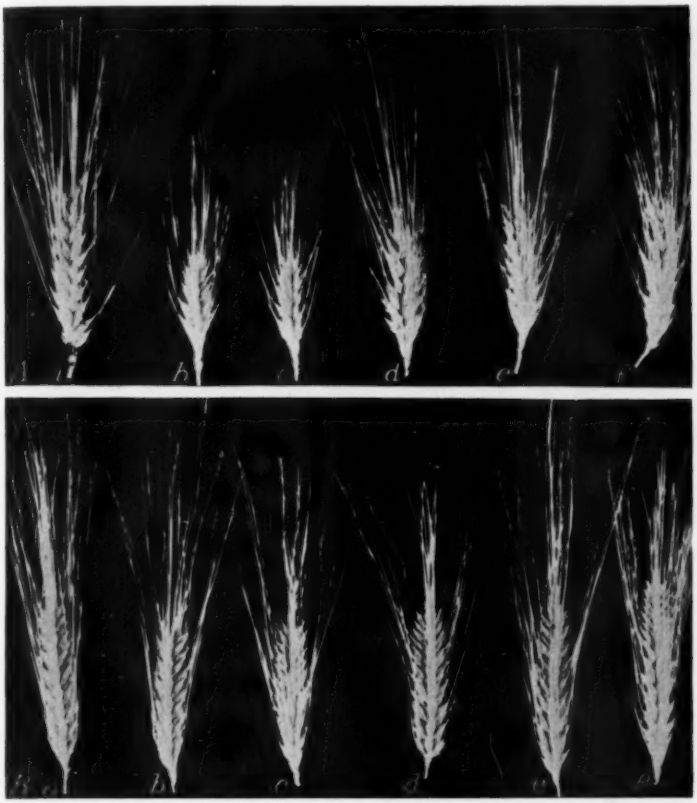

FIGURE 2.—Head development in two barley varieties, (*A*) Pannier (susceptible) and (*B*) Khanaka (resistant). Inoculated with stripe rust at the following stages: *a*, Noninoculated control; *b*, seedling; *c*, three-leaf; *d*, jointing; *e*, booting; and *f*, anthesis; and reinoculated at 13-day intervals until mature.

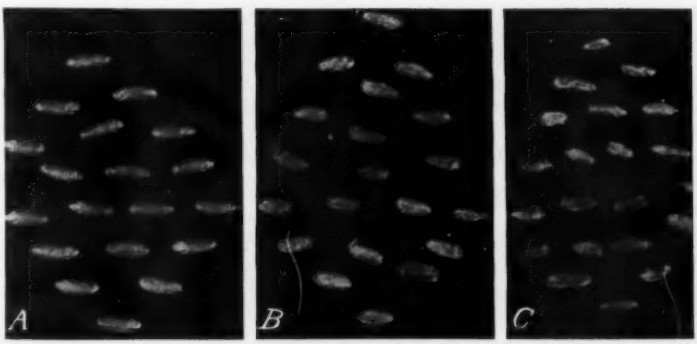

FIGURE 3.—Effect of stripe rust infection on kernel characters of a susceptible variety of wheat: *A*, Normal; *B*, moderately infected; *C*, severely infected.

In general, the roots appear to have been affected more than any other plant part, the reduction as compared with the control being 87.6 percent in the susceptible wheat variety and 75.9 percent in the susceptible barley variety inoculated in the one-leaf, seedling stage. The reduction from inoculating in the three-leaf stage was nearly as great. In this connection it should be remembered that the experiments with wheat and barley were conducted in different years and in somewhat different periods of the year, and hence to that extent are not strictly comparable. Nevertheless, the results secured for the two crops are strikingly similar, as illustrated by the reduction in roots, and as will later be shown for other plant parts as well.

There was also a heavy reduction in yield of grain, approximately 60 percent for the susceptible varieties when inoculated in the two early stages of development and nearly as much in wheat when inoculated in the jointing stage. The effect on the straw was considerably less, the reduction in yield for the susceptible wheat variety inoculated at the first three stages being above 30 percent. The reduction for the susceptible barley variety inoculated at the first two stages was about 20 percent and at the jointing stage 11.5 percent.

That the vegetative development of the plant was adversely affected is shown in table 2. The height of plant of the susceptible varieties was materially reduced, as was also the length of the head. The maturity of the plants was delayed, especially that of the wheat plants. The relatively small effect on the barley was possibly related to the fact that, because of later seeding and the use of earlier maturing varieties, the total period of growth was much less than for the wheat. The number of tillers was increased in all cases except when the plants were inoculated at anthesis, but the effect was not marked as compared with other plant parts. Many of these were supernumerary tillers that appeared after the plants had nearly matured. They were very small and weak and produced no heads.

The number of heads was materially reduced for the susceptible wheat inoculated at the first three stages and slightly reduced for the susceptible barley inoculated at the first two stages (table 2). The total number of kernels was greatly reduced, with the exception of the resistant barley, as was also the size of kernels, as shown by the number per gram of grain. This reduction in size was brought about by kernel shrinkage.

It is of special interest to note that the rust materially affected the resistant as well as the susceptible varieties, even though there was very little visible infection (table 1). Thus, early infection reduced the amount of roots by about 25 percent both in the resistant wheat and the resistant barley, and the yield of grain a like amount in the resistant wheat. In general, Khanaka, the resistant barley, was affected less than Garnet, the resistant wheat.

EFFECT OF STRIPE RUST ON AMOUNT OF WATER USED

The percentage changes, with respect to the total quantity of water used and the ratio of this quantity to the total amount of dry matter and of grain, as compared with the noninfected controls, are given in table 2.

It will be observed that the total quantity of water used was, except for the resistant wheat, slightly reduced by the stripe rust. This was obviously due to the very great reduction in growth and size of the plants, as previously noted. Also, no doubt partly as a result of this reduced growth, the amount of water used per unit of dry matter and of grain was greatly increased except for the resistant barley inoculated at the boot and anthesis stages. As with other determinations, the results for the susceptible wheat and barley are very similar. Thus, for the inoculations in early stage of development, the amount of water used per unit of dry matter was more than doubled (110 percent) for the susceptible wheat and nearly doubled (89 percent) for the susceptible barley. The effect on the grain was even greater, owing, no doubt, to the relatively greater reduction in yield of grain by the disease. It is of interest to note that the amount of water used per unit of dry matter and of grain was increased also for the resistant wheat variety.

It has been noted that the total quantity of water used per jar or per plant was nearly as great for the rusted as for the nonrusted plants, although the growth of plants, as indicated by the total dry matter produced, was very much less. This suggests that the loss of water per unit area of plant surface was considerably greater for the rusted plants than for the controls.

SUMMARY AND CONCLUSIONS

Two varieties of spring wheat, Chogat (C. I. 6244) and Garnet (C. I. 8181), susceptible and resistant, respectively, and two varieties of spring barley, Pannier (C. I. 1330) and Khanaka (C. I. 743), susceptible and resistant, respectively, were grown to maturity in sealed containers in the greenhouse for the purpose of studying the effect of stripe rust (*Puccinia glumarum*) infection, initiated at various stages of plant development, on growth, yield, time of heading and ripening, on various plant characters, and on the water economy of the host plant. The two crops were grown in different years and during somewhat different periods of the year, and so are not directly comparable. However, the results were strikingly similar.

Infection in early stages of growth greatly reduced and retarded the growth of the plants, reduced the production of roots, the yield of grain and straw, the height of the plants, the size and number of heads, and the size and number of kernels. Thus, for infection in the one-leaf, seedling stage, the reduction for the weight of roots of the susceptible wheat and barley was 87.6 and 75.9 percent, respectively; for the production of total dry matter, 55.7 and 48.7 percent; and for the yield of grain, 65.1 and 64.5 percent. The quantity of water used per plant was slightly reduced, but, because of the much greater reduction in growth, the quantity per unit of dry matter and of grain was greatly increased.

The effect of the rust was much less as inoculation was delayed. Even so, there was a material reduction in weight of roots and grain in the susceptible varieties with inoculation as late as anthesis. The amount of injury appeared to be related primarily to the time of inoculation with reference to the stage of development of the host, rather than to the amount of rust that developed, since the latter was much the same for each inoculation group. The reduction in

yield of grain was due to a decrease in the size and number of heads per plant, the size and number of kernels, and the shriveling of the kernels.

Infection had a similar though less effect on the resistant varieties, especially the wheat, even though very few rust pustules appeared on the plants. Thus, the weight of roots of the wheat was reduced 24.5 percent; that of the dry matter, 18.2 percent; and that of the grain, 31 percent. The effect on the resistant barley was somewhat less.

The results of these experiments are strikingly similar to those reported by Johnston and Miller (*7*) for leaf rust of wheat. Not only are the general effects much the same but the percentage changes brought about as a result of infection are in many cases very similar. As in the present case, the plant organs most severely affected and apparently the most sensitive were the roots. Likewise, the development of the plant above ground, the yield of grain, and the water economy were affected in much the same way. The evidence of injury by leaf rust on the resistant variety used by Johnston and Miller is less convincing than in the present case, though it seems likely that both rusts are capable of causing a loss even in resistant varieties. The effect on the kernels was much the same as that recorded for leaf rust on wheat by Caldwell et al. (*2*) and by Johnston and Miller (*7*), except that stripe rust appears to shrivel the kernels as well as to reduce their size, whereas leaf rust only reduces the size.

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THE RELATIVE QUANTITIES OF OIL DEPOSITED UPON PARAFFIN-COATED PLATES AND UPON PLANT FOLIAGE BY OIL SPRAYS [1]

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INTRODUCTION

The insecticidal and "phytocidal" effects of oil emulsions are largely controlled by the quantity of oil deposited upon the sprayed surfaces. Since a knowledge of the concentration of oil in a spray by no means affords an accurate basis for comparing different sprays, Cressman and Dawsey (*2*) [2] studied insect mortality from the standpoint of deposit as measured directly from the plant. Rohrbaugh (*9*) also compared plant response directly with measured oil deposits. While the final criterion of spray efficiency is the degree of insect kill together with absence of plant injury, there is need for a more rapid means for preliminary evaluation of emulsions.

Smith (*10*) and English (*5*) were the first to propose measurement of oil dosage in gaging the efficiency of oil sprays. Other investigators (*1, 4, 7, 11, 12, 13*) have more recently turned their attention toward devising ways of estimating the quantities of oil retained on plants or certain types of surface, such as glass, beeswax, or paraffin. At present accurate determination of oil on foliage is limited to petroleum oils. Some disadvantages of measuring oil on foliage are the time required, the presence of lesser quantities of natural leaf waxes or oils, and such factors as inequalities of plant surface or nature of the solvent used in extraction. Schemes for spraying surfaces of glass or wax-covered plates have won favor because of the ease of weighing and calculating the oil deposit. The plate methods are not restricted as to the nature of the oil. In spite of these advantages, however, their usefulness is open to question inasmuch as very few data have been published regarding the relative deposits produced on plate surfaces and plant surfaces.

The studies recorded here were undertaken in an effort, first, to develop a suitable technique capable of giving reproducible plate deposits, and then to determine whether a definite proportionality exists between the quantities of oil retained by the plate surfaces and a selected plant surface.

DESCRIPTION OF SPRAYING APPARATUS

The laboratory sprayer (fig. 1) consisted of an aluminum pressure cooker of 8 liters' capacity housing a churn agitator driven at a speed of 340 revolutions per minute, an air-inlet tube to admit compressed air at a pressure of 40 pounds per square inch, and an adjustable vermorel-type nozzle with disk opening of 1.16 mm diameter.

[1] Received for publication Oct. 17, 1936; issued April, 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 398.

This nozzle was adjusted to deliver a solid cone of spray at the rate of 1 liter per minute. Both plants and plates were sprayed with this apparatus under the same conditions with regard to tank pressure, quantity of liquid initially placed in the tank, speed of agitation, adjustment of nozzle, etc. Seven liters of fluid were placed in the tank at one charging.

The plates were mica disks that had been dipped in hot paraffin and allowed to cool at room temperature. The area of each disk was 200 cm², including both faces. Such disks had several advantages over glass plates. They could be easily cut from a sheet of mica with a pair of scissors, and the weight was much less than that of window glass of the same area. The mica possessed greater flexibility and

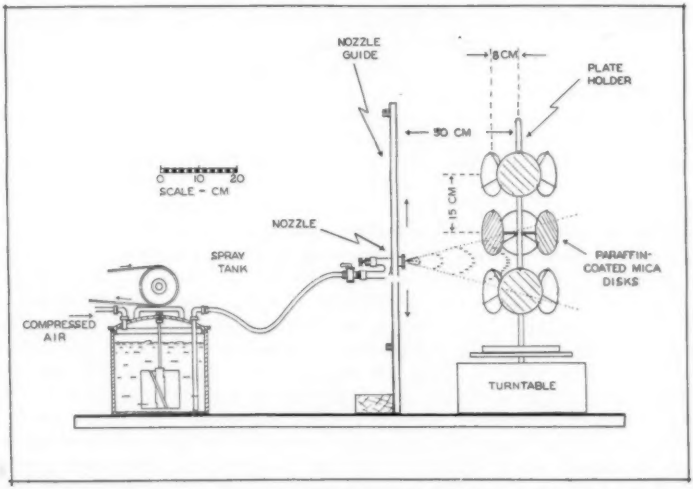

FIGURE 1.—Laboratory spraying apparatus, showing method of application to the paraffin-coated mica plates. Potted plants may also be sprayed by placing the pot on the turntable in the position occupied by the plate holder.

resistance to heat when dipped in the paraffin coating bath to produce a new clean surface each time.

The plate holder consisted of a laboratory ring stand to which wires were soldered to allow a separate three-point mounting for each plate. There were three rows of plates with three plates per row, so that the holder accommodated nine plates with a total area of 1,800 cm². The distance from the ring-stand rod to the center of the plates was 8 cm, while that from the spray nozzle to the rod was 30 cm. When placed upon the phonograph turntable, the holder revolved at the rate of 20 revolutions per minute.

SPRAYING THE PLATES AND PLANTS

During applications to the plates the nozzle was fixed so that it might be moved up and down by hand in a vertical plane, thus directing the stream horizontally toward the revolving mechanical "plate tree." The operation closely simulated the spraying of one of the potted plants. Each set of plates was sprayed for 1 minute.

The plate deposit was obtained by subtracting the weight of a set before application from the weight after evaporation of the aqueous portion of the spray liquid. The handling and weighing of a set of nine plates was accomplished in the later tests by means of a special rack constructed so that it might be hooked on the beam of the balance. In the experiments reported in tables 1 and 2, and in earlier tests, the plates were weighed separately to obtain the additional information as to the variations in deposit on individual plates. The sprayed plates were dried in a cabinet type of desiccator over concentrated sulphuric acid. With this procedure only about 5 hours was required as compared with 24 hours or more, depending upon the prevailing humidity and temperature, when dried outside.

One tank containing seven liters of spray liquid was sufficient to spray, first, two potted plants, then the set of nine plates, and later three more potted plants. By applying the spray in this order both plants and plates received dosages under comparable conditions and from the same tankful of liquid.

Potted chrysanthemum plants, grown in the greenhouse, which had been allowed to grow to a height of more than 12 inches, but which had been trimmed to uniform sizes between 10 and 12 inches, were sprayed to furnish foliage material for the determination of the oil retained on the plants. During most of the spraying operations each plant was placed upon the revolving turntable, and while the nozzle was being manipulated by hand entirely, the spray was directed at the stems for 24 seconds, over the upper surfaces of the leaves for 15 seconds, and then, after the pot had been lifted from the turntable, on the under sides of the leaves during the last 21 seconds. The plants were set aside and not moved until the water had evaporated. This procedure with the plants was followed in all the experiments except those recorded in table 1, in which they were sprayed in a manner identical with that used on the "plate tree."

The oil deposits on the plants were determined according to a method recently developed by the senior author (*3*), in which leaf disks 1 cm in diameter were clipped from the plants in numbers depending upon the oil content of the leaves.

PREPARATION OF SPRAYS

OIL SPECIFICATIONS

A highly refined, water-white petroleum oil, testing 94.9 seconds viscosity (Saybolt at 100° F.), 94.0 percent unsulphonatable residue, 0.25 percent volatility (24 hours' heating at 100°), and 0.8512 specific gravity (20°/20° C.), was used in the preparation of all emulsions.

EMULSIFICATION

Concentrated emulsions were prepared with the following proportions of ingredients:

(1) Ground-Bone Glue

Three hundred cubic centimeters of petroleum oil and 300 cc of aqueous solution containing 18 g of glue; emulsified in 1.5-liter beaker by stirring for 20 minutes with a high-speed drink mixer of the drug-store type; made up to 1,500 cc with distilled water to give a 20-percent oil concentrate.

(2) Potassium Fish-Oil Soap

Formula A: 300 cc of petroleum oil and 300 cc of aqueous solution containing 36 g of potassium fish-oil soap of 35-percent soap content; emulsified as in (1) but stirred for only 10 minutes. Formula B: 300 cc of petroleum oil and 100 cc of aqueous solution containing 12 g of 35-percent potassium fish-oil soap; stirred 10 minutes, then 200 cc of aqueous solution containing 24 g of 35-percent potassium fish-oil soap added; diluted as in (1).

(3) Ammonium Caseinate

Three hundred cubic centimeters of petroleum oil and 300 cc of aqueous solution containing 1.08 g of casein and 0.75 cc of 28-percent ammonium hydroxide;[3] emulsified as in (2), formula A.

(4) Bentonite

Three hundred cubic centimeters of petroleum oil and 300 cc of aqueous suspension containing 18 g of bentonite; emulsified as in (2), formula A. Successive batches were prepared the day before application and stored in a large glass bottle, the number depending on the quantity of stock needed. Distilled water was employed throughout except with the bentonite, where tap water was used, since hard water is known to facilitate its emulsification.

COMPARISON OF OIL DEPOSITS ON PLANTS AND PLATES

Several series of sprays were applied to both plants and plates for comparison of the oil deposits produced on the two types of surface. Sprays containing bone glue as the emulsifier were used in two series, and one series each contained potassium fish-oil soap, ammonium caseinate, and bentonite. In each series there were five different concentrations of oil ranging from 1 to 5 percent by volume.

BONE-GLUE EMULSIONS

The quantities of oil deposited on the nine mica plates and on four foliage samples, together with the calculated means, when bone glue was used as the emulsifier, are shown in table 1. The mean plate deposits were calculated after the weights of the glue residues had been subtracted from the total deposits. The glue deposit from the 4-percent oil spray was determined experimentally by spraying a set of nine plates with an equivalent glue solution. The value found was 1.66 mg per plate. Appropriate corrections were also applied to the other oil concentrations. It may be observed that the plate deposits increased with the concentration of the oil in the spray, as was to be expected. The mean plant deposit also increased with spray concentration, but the plant deposits were always larger than the corresponding plate deposits. The ratios of the plant deposits to corresponding plate deposits showed no definite trend over the range of spray strengths chosen.

[3] These are the proportions recommended by Newcomer and Carter (*8*)

TABLE 1.—*Comparison between the quantities of oil deposited upon chrysanthemum foliage and upon paraffin-coated plates by oil sprays containing bone glue as the emulsifier, when spraying was from the horizontal direction only*

PLATE TESTS

Oil in spray (percent)	Oil found per plate— 1	2	3	4	5	6	7	8	9	Mean plate deposit per square centimeter×10³
	Mg	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Cc*
1	10.1	8.6	7.6	8.7	9.9	9.5	8.4	9.0	6.7	4.9
	11.2	9.1	8.6	7.4	9.2	8.6	8.5	7.3	8.9	4.9
	8.8	8.6	9.6	8.8	7.5	7.8	9.0	6.3	8.0	4.6
2	16.8	13.9	19.5	15.0	19.6	16.1	18.4	15.2	------	9.4
	15.4	15.3	14.8	15.1	13.7	15.9	16.1	15.5	14.0	8.4
	14.3	13.0	12.6	14.1	13.2	12.5	12.3	13.2	14.3	7.3
3	19.2	16.4	18.6	23.6	22.6	21.3	19.1	21.7	22.6	11.4
	21.9	22.4	25.5	21.8	22.8	20.0	20.9	20.4	18.7	12.0
	20.9	24.2	25.4	21.0	21.9	18.5	23.3	19.2	22.5	12.1
4	30.7	30.6	30.8	28.5	30.3	28.8	25.8	27.7	28.2	16.1
	23.5	23.5	29.2	30.1	23.4	26.7	30.2	25.5	26.3	14.6
	23.4	25.2	24.5	30.7	22.9	28.2	24.7	26.0	27.0	14.2
5	39.8	31.6	35.5	34.1	44.4	34.8	39.5	42.0	35.7	20.8
	35.7	33.1	38.7	35.7	35.2	37.2	33.4	36.8	35.0	19.8

PLANT TESTS

Oil in spray (percent)	Leaf disks per sample	Oil found per sample×10³— 1	2	3	4	Mean plant deposit per square centimeter×10³	Ratio of plant deposit to plate deposit
	Number	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	
1	200	32.2	32.2	30.9	------	10.1	2.06
		43.3	39.8	41.2	35.0	12.7	2.59
		35.5	38.4	39.2	34.7	11.8	2.57
2	200	65.8	62.5	64.0	------	20.4	2.17
		49.1	60.9	51.2	57.8	17.4	2.07
		56.9	59.0	55.6	55.5	18.1	2.48
3	150	66.1	65.0	81.0	76.0	30.6	2.68
		62.1	74.3	70.8	------	29.3	2.44
		70.9	68.8	65.5	65.0	28.7	2.37
4	150	95.8	88.1	106.4	81.4	39.4	2.45
		86.9	80.3	70.6	73.7	33.1	2.27
		98.4	77.5	75.5	76.3	34.8	2.45
5	100	86.2	77.1	67.4	81.8	49.7	2.39
		79.8	------	66.2	59.8	43.7	2.21
Mean							2.37

When the mean plant deposits are plotted against the mean plate deposits, as has been done in figure 2, a straight line fits the experimental values very well. It is of course evident that when the plant deposit was zero the plate deposit was zero, and this would correspond to the experimental condition where the oil concentration in the spray was also zero. Thus, the origin has been assumed to be the most reliable point for the curve to pass through. The slope of the line was taken as the mean of the ratios of plant deposit to plate deposit.

It was concluded from these data that the plant deposits were about 2.4 times as large as the plate deposits, and this relation was independent of the spray concentration under the experimental conditions.

The foregoing sprays were applied to the plants in a manner identical with that employed in spraying the "plate tree"; that is, the potted plants were set upon the turntable and sprayed one at a time, the nozzle guidepost being used as illustrated in figure 1. In spraying potted chrysanthemum plants infested with mealybugs, however,

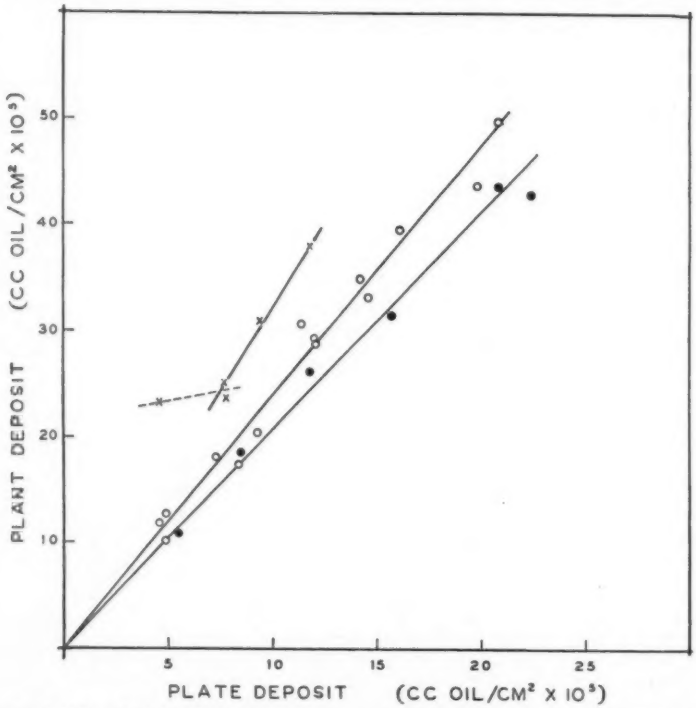

FIGURE 2.—Mean plant deposits plotted against mean plate deposits for oil sprays emulsified with glue (first series, circles; second series, dots) and potassium fish-oil soap (crosses). Plotted from data in tables 1, 2, and 3.

it had been demonstrated before the present investigation was undertaken that such a simple method would not suffice where it was necessary to direct the stream from several different angles toward the stems, upper surfaces, and under surfaces of the leaves. In the remaining experiments, therefore, the plants were sprayed from the three different angles mentioned rather than from the single horizontal direction. To make certain that the change in the method of spraying did not unduly alter the results, a second series of glue emulsions was applied by this method. The data from this second series are shown in table 2 and plotted in figure 2. The ratios of plant deposit to plate deposit were again substantially constant, with a mean of 2.06 as compared with 2.37 in the first set of sprays.

TABLE 2.—*Comparison between the quantities of oil deposited upon chrysanthemum foliage and upon paraffin-coated plates by oil sprays containing bone glue as the emulsifier, when the plants were sprayed from three different angles*

PLATE TESTS

Oil in spray (percent)	Oil found per plate—									Mean plate deposit per square centimeter×10³
	1	2	3	4	5	6	7	8	9	
	Mg	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Cc*
1	10.9	9.7	11.1	11.4	9.7	8.5	8.9	8.5	8.6	5.5
2	15.7	13.6	15.7	15.0	12.4	17.0	14.8	18.1	16.0	8.5
3				22.4	19.8	20.5	26.6	17.0	21.0	11.7
4	26.6	27.2	30.4	27.6	26.7	32.7	28.0			15.7
5	38.5	30.9	42.0	41.2	44.3	38.7	36.7	42.1	46.5	22.4
	41.7	37.6	37.5	34.7	42.0	39.9	37.2	40.2	26.6	20.8

PLANT TESTS

Oil in spray (percent)	Leaf disks per sample	Oil found per sample×10³—				Mean plant deposit per square centimeter×10³	Ratio of plant deposit to plate deposit
		1	2	3	4		
	Number	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	
1	200	33.3	32.3	33.9	36.3	10.8	1.96
2	200	58.3	60.2	58.9	55.0	18.5	2.18
3	150	62.9	63.5	54.7	65.3	26.1	2.23
4	150	71.5	79.9	71.9	72.2	31.4	2.00
5	100	69.2	73.2	65.2	61.7	42.8	1.91
		69.1	67.1	55.1	82.3	43.5	2.09
Mean							2.06

On testing the significance of the difference between these two mean ratios according to the method given by Fisher (6, *p. 120*), it was found that $t=3.60$ and $P<0.01$, showing that the second ratio was significantly lower than that obtained in the first series.

Information as to the variability in plate deposits was obtained from the data in tables 1 and 2 by analyzing the variances of all deposits resulting from applications of identical oil concentrations. From the standard error thus obtained, the difference between two means required to give odds of 19 to 1 in favor of significance was calculated on the assumption that each mean was based on nine plates. These differences, expressed as percentages of the mean of all plate deposits at respective oil concentrations, were as follows:

Oil concentration (percent):	*Difference (percent)*
1	12.5
2	9.5
3	11.3
4	8.5
5	10.6

From these figures it is evident that the variability was proportional to the mean deposit and that a difference of approximately 10 percent of the mean deposit was necessary for two means to differ significantly.

At the lower oil concentrations the leaf samples contained more disks than the plates; consequently, the variability of results with the

plant deposits would be expected to be less at the lower concentrations. Analyses similar to the above showed that at oil concentrations of 1 and 2 percent a difference of about 10 percent in mean plant deposits should be considered significant. A greater difference was found necessary at the 3-percent concentration, while in the four sprays applied at a concentration of 4 percent the difference required for significance was about 22 percent of the average deposit.

POTASSIUM FISH-OIL SOAP EMULSIONS

The fish-oil soap gave sprays with properties quite different from those of the glue emulsions even though the same petroleum oil was used. The same technique was employed as in the second glue series, except that all spray concentrations were prepared by appropriate dilutions from the same batch of stock mixture to eliminate the possibility of variation in distributions of oil-droplet size. Table 3 lists the plate and plant deposits obtained with the spray containing potassium fish-oil soap prepared according to formula A. The plates were weighed collectively and the quantity of oil deposited thereon was determined by subtracting the weight of soap present from that of the total residue (oil plus soap). The soap residue on the plates was determined by spraying a set of nine plates with a soap solution equivalent to that contained in the 4-percent oil spray, and the residue for the other sprays was calculated from this single experimental value. The last four oil concentrations gave substantially constant ratios, but the 1-percent spray gave a ratio well outside the limits of error.

TABLE 3.—*Comparison between oil deposits on plant foliage and on paraffin-coated plates from sprays containing potassium fish-oil soap (formula A) as the emulsifier*

PLATE TESTS

Oil in spray (percent)	Deposit per 9 plates			Mean plate deposit per square centimeter $\times 10^3$
	Total	Soap	Oil	
	Mg	*Mg*	*Mg*	*Cc*
1	72.3	1.5	70.8	4.6
2	120.9	2.9	118.0	7.7
3	124.0	4.4	119.6	7.8
4	149.4	5.9	143.5	9.4
5	187.3	7.4	179.9	11.8

PLANT TESTS

Oil in spray (percent)	Leaf disks per sample	Oil found per sample $\times 10^3$—				Mean plant deposit per square centimeter $\times 10^3$	Ratio of plant deposit to plate deposit
		1	2	3	4		
	Number	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	
1	200	73.3	72.5	82.6	62.6	23.1	5.02
2	150	75.3	49.2	60.3	51.1	25.0	3.25
3	150	60.8	57.7	50.4	53.8	23.6	3.03
4	100	47.3	48.9	48.2	50.1	30.9	3.29
5	100	58.9	58.5	62.8	57.6	37.8	3.20

Figure 2 shows the position of the various points obtained by plotting these data. The main part of the curve was obtained by drawing a straight line through the origin with a slope equal to the mean ratio for the last four spray concentrations.

The ratios obtained with sprays prepared according to formula B were smaller than those obtained with formula A, but the results paralleled those given in table 3 in that the 1-percent spray gave a ratio out of line and considerably larger than the remaining spray concentrations in the series.

Since potassium fish-oil soap emulsions do not give ratios that are constant over all concentrations, at least under the conditions of these experiments, it would not be practical to use such a plate method for standardizing these sprays on the basis of the quantity of oil retained by the plates. Comparison with the glue-emulsion sprays applied under the same conditions shows, furthermore, that entirely different ratios were obtained with the two emulsifiers; so it would not be practicable to compare the relative insecticidal values of the two types of emulsions, when applied to plants, merely by determining the relative deposits produced on paraffin-coated plates.

Additional spray experiments with emulsions prepared with triethanolamine oleate as the emulsifier gave confirmatory results. These soap sprays were made by dilution from different batches of stock material, and the ratios obtained were even more erratic.

AMMONIUM CASEINATE AND BENTONITE EMULSIONS

The plate and plant deposits obtained when ammonium caseinate was used as the emulsifier are given in table 4, and those obtained with bentonite are shown in table 5. Both these series were carried out in the same manner as the potassium fish-oil soap series and the second glue series, corrections always being made for the emulsifier

TABLE 4.—*Comparison of deposits on plant foliage and paraffin-coated plates from sprays containing ammonium caseinate as the emulsifier*

PLATE TESTS

Oil in spray (percent)	Deposit per 9 plates			Mean plate deposit per square centimeter $\times 10^5$
	Total	Caseinate	Oil	
	Mg	*Mg*	*Mg*	*Cc*
1	143.6	0.8	142.8	9.3
2	238.0	1.6	236.4	15.5
3	314.2	2.3	311.9	20.4
4	353.6	3.1	350.5	22.9
5	447.2	3.9	443.3	29.0

PLANT TESTS

Oil in spray (percent)	Leaf disks per sample	Oil found per sample $\times 10^3$—				Mean plant deposit per square centimeter $\times 10^5$	Ratio of plant deposit to plate deposit
		1	2	3	4		
	Number	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	
1	200	95.7	92.0	86.4	85.8	28.6	3.08
2	200	144.8	137.8	136.5	150.6	45.3	2.92
3	150	143.4	144.1	122.8	119.4	56.2	2.75
4	50	60.3	52.4	52.9	57.7	71.0	3.10
5	40	54.7	54.2	65.6	63.6	94.7	3.27
Mean							3.02

deposit on plates. Both gave substantially straight-line relationships between the plant deposits and the corresponding plate deposits.

The data from tables 4 and 5 have been plotted in figure 3. The mean ratio of plant deposit to plate deposit for the ammonium caseinate sprays was 3.02, while that for the bentonite sprays was 9.32. In absolute magnitudes the ammonium caseinate sprays gave much larger deposits than the glue sprays, and the deposits from the bentonite sprays were extremely high. Thus, the bentonite spray of 1-percent oil concentration gave a foliage deposit of 86.6×10^{-5} cc of oil per square centimeter, while the glue spray at 5-percent oil concentration gave a deposit of only 43.5×10^{-5} cc of oil per square centimeter, or just half as much when it was at five times the concentration of the bentonite mixture.

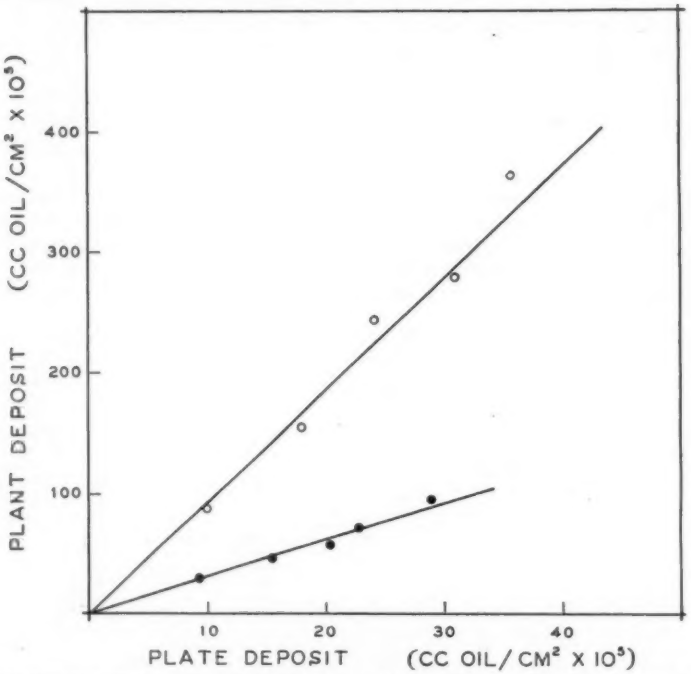

FIGURE 3.—Mean plant deposits plotted against mean plate deposits for sprays containing ammonium caseinate (dots) and bentonite (circles). Plotted from data in tables 4 and 5.

DISCUSSION

The results obtained with various types of emulsions as described above leave considerable doubt as to the practical usefulness of the paraffin-plate method in standardization of sprays. In these tests conditions of emulsification, preparation of plates, and application to plants and plates were carefully controlled, the variables being the type of emulsifier and the oil concentration. Nevertheless, no two mean ratios of plant deposit to plate deposit were the same in any set of sprays. In the case of the emulsions stabilized with soaps, even a

change in the oil concentration accompanied by change in emulsifier concentration in the dilute sprays caused erratic differences in the ratios within respective series. Moreover, granting that the paraffin-plate method has a limited usefulness in special cases where work may be confined to specific types of emulsifiers, the labor involved in first determining experimentally the relation between the plant deposit and the plate deposit is considerably more than simply making analyses of the oil directly from the foliage. Certainly analysis of oil on the foliage is more accurate, for it can be shown from the data in table 1 that multiplying the mean plate deposits by the average ratio of plant deposit to plate deposit leads to estimates of plant deposits differing by as much as ± 12 percent from the observed values.

TABLE 5.—*Comparison of oil deposited on plant foliage and paraffin-coated plates from sprays containing bentonite as the emulsifier*

PLATE TESTS

Oil in spray (percent)	Deposit per 9 plates			Mean plate deposit per square centimeter × 10^3
	Total	Bentonite	Oil	
	Mg	*Mg*	*Mg*	*Cc*
1	157.4	4.9	152.5	10.0
2	284.7	9.8	274.9	18.0
3	385.2	14.5	370.7	24.2
4	493.9	19.4	474.5	31.0
5	570.1	24.2	545.9	35.7

PLANT TESTS

Oil in spray (percent)	Leaf disks per sample[1]	Oil found per sample × 10^3—				Mean plant deposit per square centimeter × 10^3	Ratio of plant deposit to plate deposit
		1	2	3	4		
	Number	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	
1	30	45.8	37.0	39.6	40.8	86.6	8.66
2	30	79.8	70.5	70.4	78.6	155.5	8.64
3	20	80.8	75.2	73.0	77.2	243.6	10.07
4	15	73.6	69.1	60.9	60.2	279.4	9.01
5	15	91.4	94.8	67.8	89.7	364.0	10.20
Mean							9.32

[1] Initially there were 100 leaf disks per sample, but after extraction the residues were dissolved in a mixture of alcohol and carbon tetrachloride and all made up to 100 cc in volumetric flasks; aliquot portions were then pipetted out for analyses in the Babcock bottles. The figures in this column, therefore, also express the number of cubic centimeters in respective aliquots.

The four types of emulsions investigated were picked at random as being representative of mixtures that might be used commercially. It appears probable that if no systematic relationship exists between plant and plate deposits when a single oil is being used, the plate method would be no more reliable when a number of different oils were being used in combination with the emulsifiers.

In spite of the fact that the described plate method does not appear very promising for estimation of oil deposit on plants, there are several successful uses to which plates have been put at this laboratory. For example, they have been employed in studying the rate of disappearance of spray residue as well as the mechanics of spray deposition.

SUMMARY

A method is described for spraying paraffin-coated mica plates with oil emulsions and determining the quantities of oil deposited by difference in weight.

Comparison between the quantities of oil deposited on the paraffin surfaces of the plates and those deposited on chrysanthemum foliage, as determined by chemical analysis, showed that various emulsions give different ratios of plant deposit to plate deposit. It is therefore not practical to attempt to standardize the sprays intended for the plant surface against the plate surface.

Within certain limits of error, emulsions containing glue, ammonium caseinate, or bentonite gave characteristic ratios of plant deposit to plate deposit which were independent of the oil concentration or the emulsifier concentration in the dilute sprays, but with soap emulsions these ratios varied with the concentration of oil and emulsifier.

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